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SOME FURTHER EFFECTS OF AN ANTAGONISTIC TEMPERATURE GRADIENT UPON THE FROG'S EGG

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FOUR TEXT FIGURES AND ONE PLATE (FOUR FIGURES)

This preliminary note deals briefly with a set of experiments carried out in Easter, 1929. Similar experiments were carried out in Easter, 1927, frogs' eggs being exposed to temperature gradients which were adjuvant (apico-basal), antagonistic (baso-apical), and side to side in relation to the egg polarity (Dean, Shaw, and Tazelaar, '28). The apparatus was similar to that used previously and consisted of two silver discs fused on to shallow copper plates which were supplied with inlet and outlet tubes through which a flow of water at the required temperature could pass. The eggs were placed between the silver plates together with small slips of glass of a definite thickness; these slips kept the silver discs a certain distance apart and prevented the eggs from being crushed. The temperature of the cold water was about 6°C. and of the warm about 24.5°C. The actual temperature difference between the two poles of the eggs could not be measured, but was probably considerable.

The chief difference between these and the earlier experiments was the greater length of time for which the eggs were under experimental conditions in the later experiments. In 1927 the longest period was eleven hours, whereas in 1929 the eggs were between the plates for at least twenty-four hours and very often for longer.

In most cases where the eggs were under experimental conditions for only twenty-four hours, the same type of result

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was obtained as in previous experiments, namely, the whole early development (as judged by cell size) of the eggs exposed to an adjuvant temperature gradient was more advanced than in those exposed to an antagonistic temperature gradient, even though all other conditions, and therefore the mean temperatures of the two sets of eggs, were similar. This even applied to the yolk cells of the former series, though these had actually been chilled. In experiments where the period was longer, however, practically all those treated with an adjuvant temperature gradient died, gastrulation appeared to be impossible, and large masses of yolk were thrust out. For this reason, in this short paper we shall deal almost entirely with those treated with an antagonistic temperature gradient; those treated with a side-to-side temperature gradient will be dealt with in a later paper.

The experimental treatment of most of the eggs was begun at the two-celled stage. After experimental conditions lasting, in most cases, for about thirty-two hours, the eggs treated with an antagonistic temperature gradient were at various stages of gastrulation. Usually the dorsal, ventral, and lateral lips had formed, so that a large rim of overgrowing tissue was present which enclosed a fairly extensive area of yolk cells. In the case of a batch of eggs treated for the same period and at the same temperature with an adjuvant temperature gradient, only the dorsal lip was well formed, but it was situated in a more ventral position and the area of yolk cells showing was often smaller.

The eggs were then placed in normal conditions. After about twenty-four hours, practically all those treated with an adjuvant temperature gradient were dead. Among the very few survivors, gastrulation was attempted, and during this process masses of yolk were extruded. In the case of those exposed to an antagonistic temperature gradient, however, a very interesting feature was invariably present in the shape of a peculiar ring-shaped groove which appeared above the equator (fig. A). This feature was not obtained in previous experiments. It was also found to be present to a much less

marked extent in fertilized eggs which were left in a cold chamber for some days where the temperature was below 4°C. The groove, which was not necessarily complete, slowly disappeared in all cases after the eggs were allowed to continue their development under normal conditions, but in the most pronounced cases the animal pole became much wrinkled. The eggs which were left in the cold chamber can be compared with eggs exposed to an antagonistic temperature gradient, by assuming that since the animal pole is more susceptible than the vegetative pole, the cell division rate at that region will be comparatively more affected than at the more inert vegetative pole, and hence a condition approximating to an antagonistic temperature gradient would be obtained. A similar condition was also produced by Vogt ('22) in Triton embryos in which a portion of the roof of the blastula was removed. An explanation of this occurrence was suggested by Vogt and elaborated by one of us (G. R. de Beer, '27), namely, that the future ring of overgrowth is by these processes (removal of blastula roof or subjection to antagonistic gradients) drawn up above the equator; when gastrulation starts, this rim attempts to grow over and at the same time to decrease its diameter, and this results in a constriction above the equator. This would imply a separation of the process of invagination from that of epiboly. This explanation might possibly account for cases in the temperature-gradient experiments in which gastrulation is not very far advanced, but does not seem applicable to cases where the blastopore is quite small and gastrulation has apparently proceeded fairly normally. On cutting sections of these forms, it was noticed that the circular groove-like invagination was internally always at the same level as the top of the yolk (fig. B) and sometimes there was a marked ingrowth of ectoderm over the yolk. It is possible that the temperature was in some cases too high or too low (in the case of those left in the cold chamber), thus having a deleterious effect upon the yolk and causing it to become more inert than usual. If, on invagination, the yolk remained immovable,

owing to its inability to divide normally and perhaps also owing to the cooled apical region retarding the later process of development, then the archenteron would be prevented from extending up as far as usual into the yolk and invagination would remain incomplete. The downgrowth of tissue from the animal pole would thus be hindered and the extra growth would naturally be pushed in at the position of weakest resistance; it would thus be forced in at the level of the top of the yolk, hence the formation of the peculiar ring-shaped groove. In a few cases the tissue did apparently grow down past the yolk in the region of the dorsal lip, and here the extra down-growing tissue is present as a fold in the dorsal lip, but on the side away from the lip the ring is present at the level of the yolk.

Later stages of these forms differed chiefly according to the degree of closure of the blastopore. Where the blastopore remained widely open, the embryo became much deformed. The most normally developing embryos of this experiment were those in which the blastopore nearly closed. In many of these forms, even where the blastopore did apparently close, the head was very small and there was a deficiency of nervous material. The brain and sense organs were often fairly well formed, but abnormally small (fig. C). Microcephaly was also obtained in the earlier experiments as the result of antagonistic gradients during segmentation, but never in any degree at all approximating that found in some of these present cases.

The forms in which the blastopore did not close were interesting to study. As the embryo lengthened so the blastopore became elongated, and at the time of the formation of the neural folds these were always found to be lying on either side of the unclosed blastopore, thus giving rise to an extreme condition of spina bifida (fig. D). Behind the anterior fold which represents the rudiment of the brain there is a deep invagination which, since it produces gill slits from its walls, must represent the foregut (fig. 1). In a stage twenty-four hours older, the foregut is more differentiated,

the liver diverticulum is present, and the stomodaeal invagination is beginning to form. Lying in front of the posterior border of the blastopore is a smaller invagination. The tail, by the time its somites are well formed, is always bent up and forward over the yolk (figs. 2 and 3). In still older embryos the yolk is still showing, although the neural folds have become somewhat drawn together. The external form

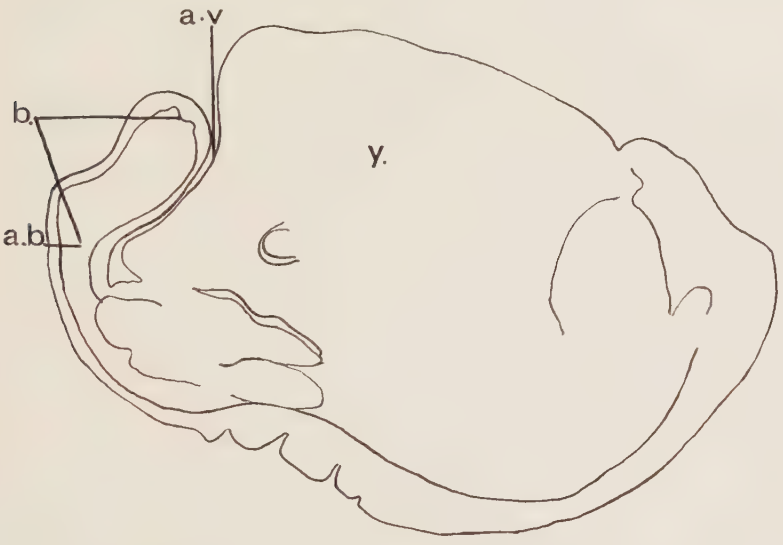


Fig. 1 Camera-lucida drawing of a longitudinal section through about the middle of an embryo treated in the same way as the embryo shown in plate 1, figure D. *a.b.*, anterior limit of brain; *a.v.*, anterior invagination; *b.*, brain; *y.*, yolk mass.

of the head is sometimes well developed to a degree which is surprising after one has seen the extreme condition of spina bifida through which the embryo has passed in earlier stages. The stomodaeal invagination is now complete and opens into the foregut invagination. Thus in these forms there is invariably a connection established between the mouth in the normal position and an aperture on the dorsal surface just behind the brain (fig. 3) and this connection persists in later embryos up to the stage at which death occurs, usually when

the body length is about 4 to 5 mm. The posterior invagination is more developed (fig. 3), and on examining serial sections the two pronephric ducts are found to open into it, hence the invagination must represent the cloaca. The original invagination here must represent the hindgut and proctodaeum, from which the cloaca is later developed. (No separate proctodaeal invagination is required, since the original invagination aperture never closes.) Thus the anus in this case is at the posterior end of the yolky mass and in front of the tail.

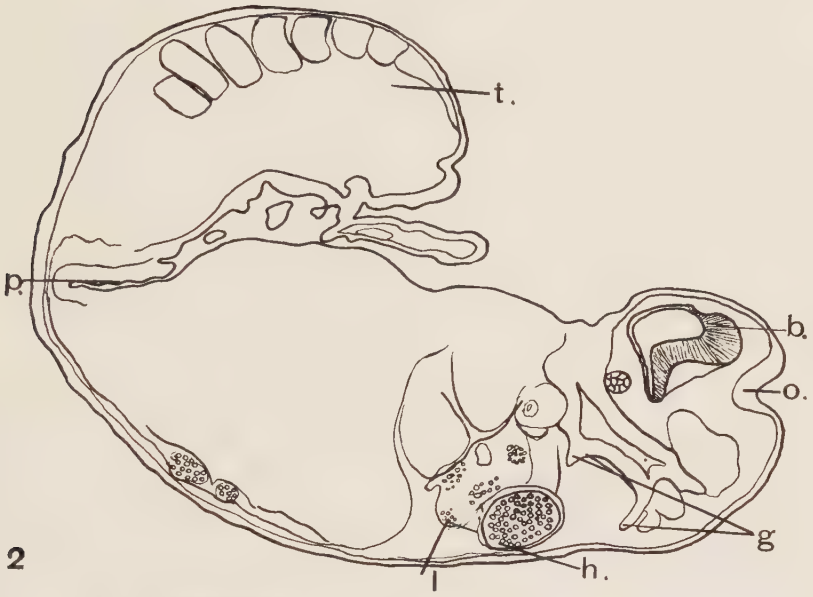
The development of the alimentary canal in these abnormal forms is interesting, since it arises by two or possibly three independent invaginations: the anterior invagination, the foregut, and the posterior invagination, the hindgut and proctodaeum. These are undoubtedly quite independent, while it is possible that the stomodaeal invagination is dependent upon the presence of the foregut. We have here a good example of independent differentiation of parts of the alimentary canal which through experimental conditions have become isolated from each other.

In all these forms treated with an antagonistic temperature gradient, the nervous system is reduced in size and in some markedly so. This can probably be accounted for in two distinct ways. First, the area of the presumptive nervous material may possibly be reduced by the application of a low temperature to the animal pole, thus preventing the cells from dividing as rapidly and to such an extent as under normal conditions. The thickness of the blastocoele roof previously mentioned in these specimens seems to bear out this possibility. There is further the point that, if the anterior end of the dorsal, foregut invagination be taken as repre-

Fig. 2 Camera-lucida drawing of a longitudinal section through an embryo treated in the same way as above, but allowed to develop under normal conditions for two days longer.

Fig. 3 Longitudinal section of same embryo through stomodaeum. *b.*, brain; *f.*, foregut invagination; *g.*, gill pouches; *h.*, heart; *l.*, liver; *o.*, olfactory invagination; *p.*, posterior invagination; *s.*, stomodaeum; *t.*, tail, bent upward and forward over yolk.

senting the position of the dorsal lip—as seems inevitable—the distance between this point and the anterior end of the brain is often considerably less than is to be expected from



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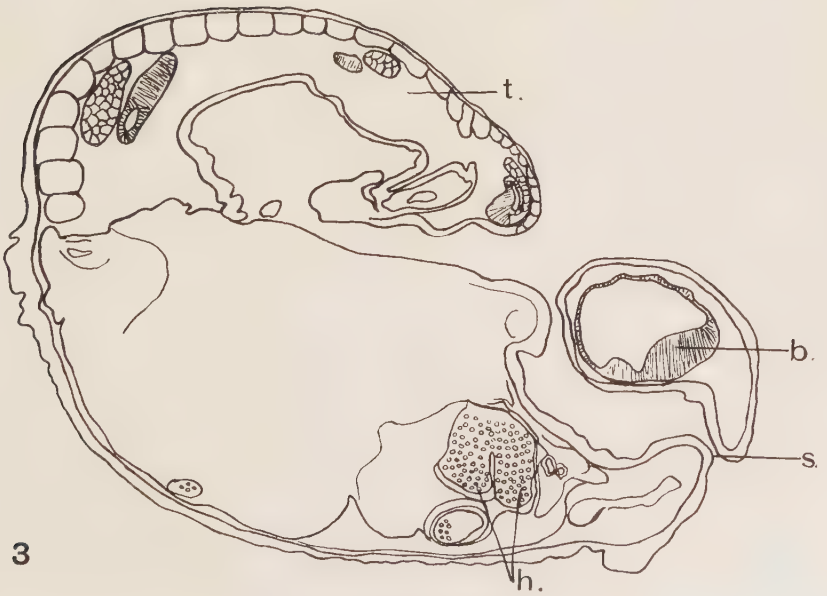

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the researches of Vogt, according to which it should comprise 80° of the egg's circumference. This presumably can be accounted for by the failure of the archenteron of these specimens to penetrate as far animalward in the yolk mass as in normal development. Since the presence of archenteron roof appears, according to the work of Spemann and his pupils, to be the final and chief determiner of medullary plate in the

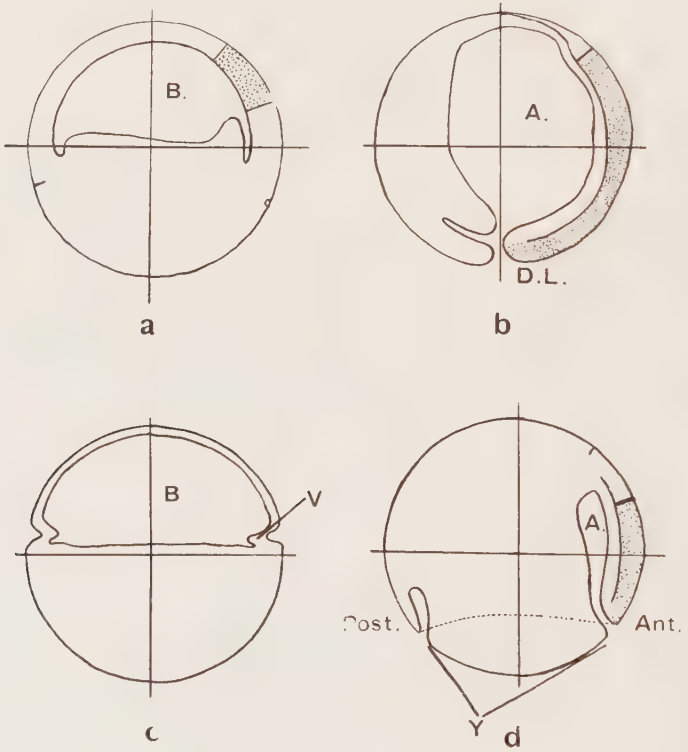

Fig. 4 To show the modified invaginatory process in the limited forward extension of medullary plate in the spina-bifida cases. a and b, after Vogt (Roux Archiv. Entwmech., 1929). Median sections through eggs of Bombinator showing: a, the prospective neural-plate material (stippled) in the blastula stage; b, formation of the archenteron and the position of the neural plate. c and d represent diagrams of what has apparently occurred in the experimental cases. c shows the ring-shaped groove in section and the thin roof of the blastocoele. d shows the limited extent of both anterior and posterior invaginations. The stippled area represents the approximate extent of the medullary plate (see text). *A.*, archenteron; *B.*, blastocoele; *D.L.*, dorsal lip; *Ant.*, anterior invagination; *Post.*, posterior invagination; *V.*, ring-shaped groove in section; *Y.*, yolk plug.

superjacent ectoderm, we should expect that in these cases the anterior limit of the brain should fall short of that of the presumptive medullary plate.

SUMMARY

Frog eggs were treated with temperature gradients for long periods (twenty-four to forty-eight hours). Previous results showing that eggs heated at the animal pole were more advanced, even as regards segmentation of the vegetative cells, than those kept at the same mean temperature, but heated vegetatively (antagonistic gradient), were confirmed.

Long-continued antagonistic gradients caused the following effects in gastrula stages: 1) A ring-shaped groove encircling the egg at the level of the top of the yolk. 2) A very thin roof to the blastocoele. 3) In later development strikingly microcephalic embryos were produced. If the closure of the blastopore is nearly complete, no other abnormality is noted. 4) Often, however, the blastopore remains widely open, producing extreme spina bifida. In these forms the foregut invagination is found dorsally just behind the brain; the proctodaeum, quite separate, dorsally just in front of the tail. The stomodaeum is later formed in the normal position, thus establishing a communication from the mouth to the dorsal surface just behind the brain. 5) In these forms it appears also that, probably owing to the incomplete extent of invagination, the most anterior section of the presumptive medullary plate does not become converted into actual medullary plate; thus the anterior end of the brain is in a different position, relative to the original egg axes, from that which it occupies in normal embryos.

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PLATE 1

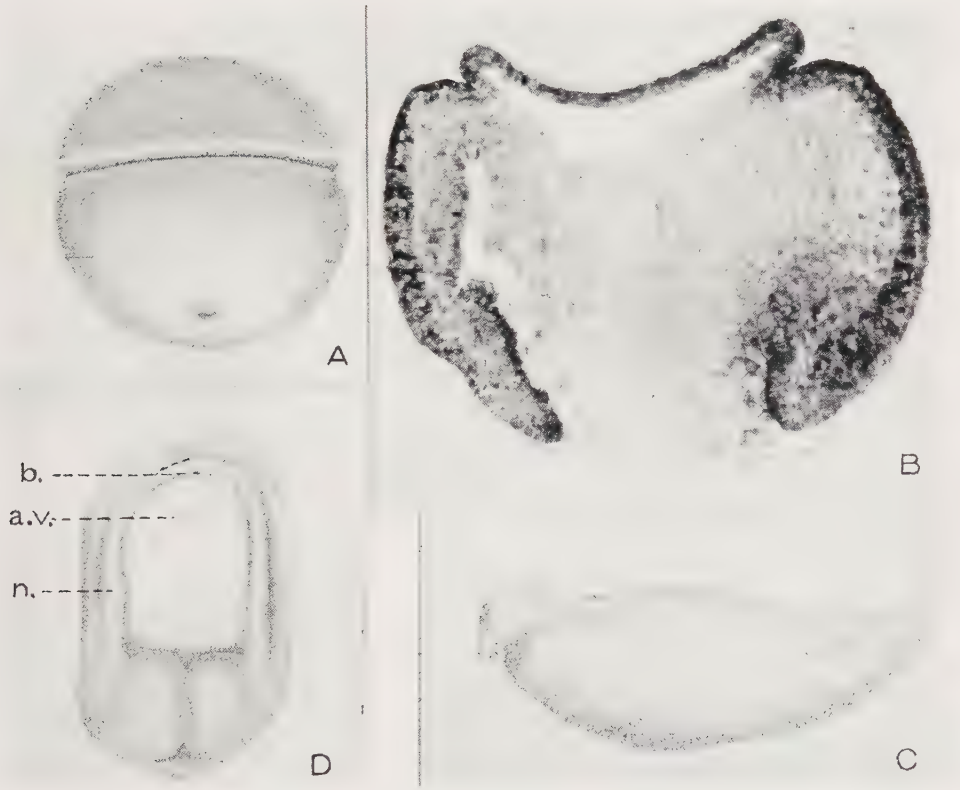
EXPLANATION OF FIGURES

A (Kc5.) Effect of an antagonistic temperature gradient on the eight-celled stage for forty-eight hours. This egg was the least affected of the batch, since the blastopore is nearly closed. Note clearly defined ring-shaped groove.

B (Kal 17/4.) Section through egg in same batch as above, but removed after only thirty-two hours. This egg was apparently more affected than the above. Note thin roof of blastocoele (collapsed in preparation), marked ring-shaped groove, and small archenteron.

C Drawing of embryo treated with an antagonistic temperature gradient for thirty-two hours, a few hours after fertilization, and then allowed to develop under normal conditions for seven days. Note extremely small head.

D (Kald 18/4.) Drawing of embryo treated with antagonistic temperature from eight-celled stage for thirty-two hours and then allowed to develop under normal conditions for forty hours. Note large, elongated blastopore. *a.v.*, anterior invagination; *b.*, anterior neural fold representing brain region; *n.*, lateral neural folds on either side of the blastopore.


A
B
b.
a.v.
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D
C

A METHOD OF COPYING BIOLOGICAL RECONSTRUCTIONS WITH ANY DESIRED FACTOR OF MAGNIFICATION

JOHN STANLEY

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THREE FIGURES

During the course of an investigation of the musculatory system of a certain gamasid mite, Laelaps echidninus, Berl., the writer had occasion to make enlarged plasticine copies of reconstructions which had been built up in the usual way from cut-out drawings of serial sections.

The method finally decided upon was a modification of one used in the copying of statuary, and both the instrument and its application will be described here in the hope that they may be of use to other workers in similar fields.

Speaking in a general way, the method consists of marking off a number of suitable points on the surface of the original, obtaining the coordinates of these points in three dimensions, and then transferring them to some other convenient region, building up the modeling material to agree with them.

If, during the process of transfer, the values of the coordinates be multiplied by any constant factor, the resultant copy will be multiplied in size by the same value.

The actual building up of the copy to fit the coordinates presents no difficulty, the necessary skill in the manipulation of the plasticine being acquired rapidly in practice.

The easy and rapid determination of the coordinates is, however, another matter, and since the truth of the copies depends upon the accurate determination of these coordinates, the instrument used must be made with some care.

The machine (fig. 1) is fairly simple in construction and is made largely from "Meccano" parts. ("Meccano" is the trade name for sets of standard metal shapes strips, wheels,

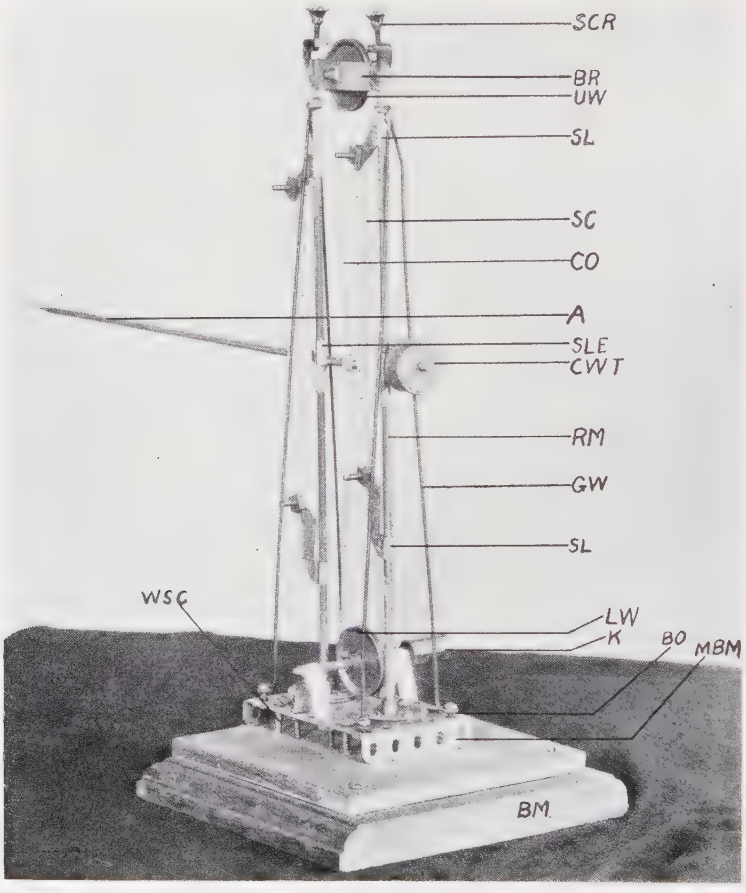


Fig. 1 View of machine for determination of coordinates, from left side. *SCR*, set-screw for adjusting tension of driving cord; *BR*, bridge supporting upper pulley wheel; *UW*, upper pulley wheel; *SL*, sliding sleeve permitting adjustment of height of scale, *SC*; *SC*, celluloid scale for evaluation of vertical coordinates; *CO*, driving cord for moving arm; *A*, pointer arm; *SLE*, sliding sleeve guiding movement of arm; *CWT*, counterweight to balance arm; *RM*, vertical rod forming track for arm; *GW*, guy wire, bracing vertical rods; *LW*, lower pulley wheel; *K*, knob for rotating lower wheel; *BO*, bolt for adjusting tension of guy wire; *MBM*, metal base; *BM*, wood base; *WSC*, wood-screw fastening metal base to wood base.

etc.—sold to boys for the making of models of machinery, etc.)

The heavy wooden base (*BM*) has two edges set accurately at right angles to each other and measures 6 inches along each edge.

A perforated "Meccano" plate (*MBM*) is fastened to it by means of four wood-screws, and two small wheels are then bolted to the plate. Three holes must be drilled in the web of each wheel for this purpose.

These wheels provide an initial support for the two vertical rods (*RM*), which are held in place by the set-screws in the hubs of the wheels. The rods are firmly braced by the guy wires (*GW*) which are soldered to them at the upper end. The lower ends of the wires are looped under the heads of bolts, as shown, so that by screwing up or down on the bolts, the tension may be varied in order to bring the rods into a vertical and parallel position.

Across the upper end of the rods is a bridge (*BR*) consisting of two plates of thin metal, with holes bored for the axle of the upper pulley (*UW*). The upper pulley together with the lower is a "Meccano" part. The axle is a short length cut from any standard "Meccano" rod. The side plates of the bridge may be cut from any standard perforated "Meccano" strip, though in this particular problem they were cut from sheet metal of similar gauge. The ends of these side plates are soldered to small metal sleeves which fit snugly over the rods.

Also, at each end of the bridge there is a small set-screw which bears on the upper end of the corresponding vertical rod. By means of these screws, the bridge may be raised or lowered to vary the tension on the cord (*CO*).

The support of the lower pulley wheel (*LW*) is self-explanatory.

The horizontal arm (*A*) is fastened to two metal sleeves (*SLE*) which fit very closely and smoothly on the rods. The accuracy of the machine depends largely on the accuracy of this fit. Hence, it is as well to have the sleeves made especially

for this purpose. One side of the cord (CO) which passes around both upper and lower pulleys is fastened to the arm at a point midway between the two vertical rods. Thus, on rotating the lower pulley by means of the knob (K), the cord, traveling around the pulleys, raises or lowers the arm, depending upon the direction of rotation.

The arm is balanced by a counterweight (CWT) which may be screwed on or off. This weight is necessary, since without it the unbalanced arm binds on the vertical rods and moves with a jerky, chattering motion.

The vertical height of the arm is determined by small celluloid scales. These also are fastened to sliding sleeves, the purpose of which will be apparent later.

In practice, a set-up is made as in figures 2 and 3. The table is covered with a sheet of graph paper, ruled in millimeters, one pair of intersecting lines near the middle of the paper being darkened with India ink. The graph paper provides the means of determining the two horizontal coordinates, the vertical one being read off from the celluloid scales of the machine.

The machine is first set up as in figure 2, with the edges of the base accurately collinear with the darkened zero lines of the paper. The arm is then raised to a convenient height, and one of the celluloid scales moved up so that its zero point coincides with the pointer on the arm. The model to be copied is then moved over so that a point on it just touches the end of the pointer. This point may then be called the 'null-point,' and its coordinates are 0, 0, 0. It is advisable to have this the lowest point on the model, thus eliminating negative vertical coordinates.

Before proceeding to copy any points, the coordinates of two other points should be found and noted down, in order that the set-up may be duplicated at any time.

To find the coordinates of any point other than the null, the pointer is raised to the desired height and the machine is then slid over the paper, so that while the sides of the base lie along lines on the paper, the tip of the pointer comes

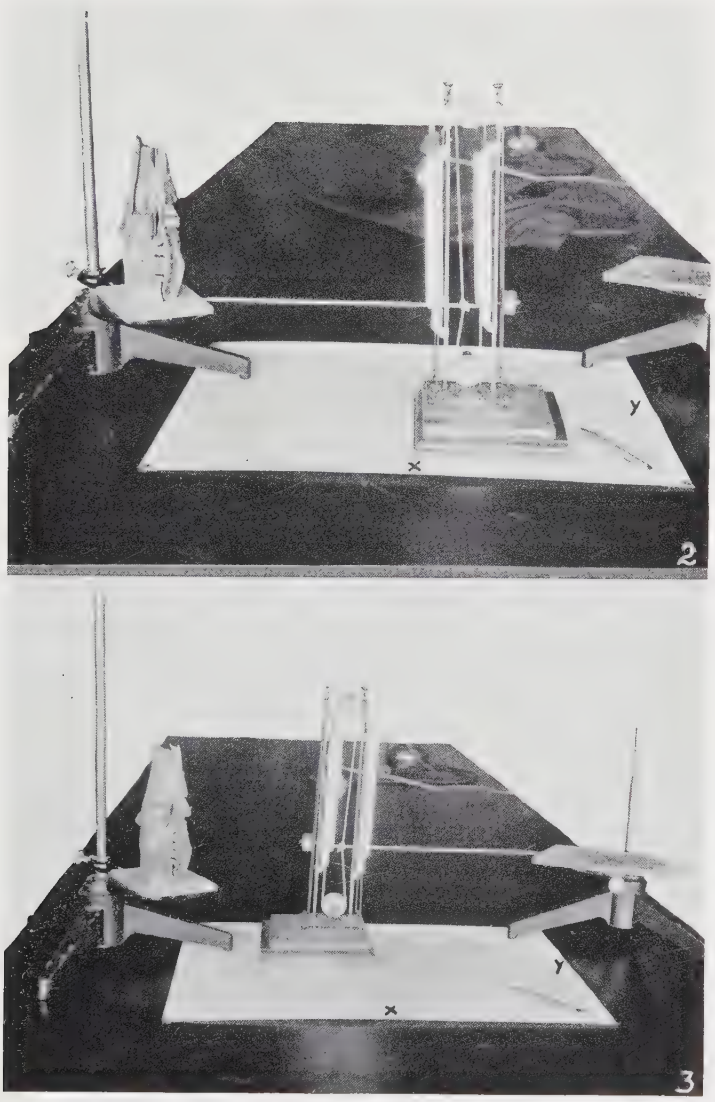


Fig. 2 Coordinate machine set up, with end of pointer on null-point of original model.

Fig. 3 Coordinate machine turned through 180°, and with pointer tip against null-point of copy to be constructed.

to touch the selected point on the model. The coordinates may then be read off at once.

It now becomes necessary to determine the null-point of the copy. The machine is set to the null-point of the original, and is then rotated around the zero point as in figure 3, the arm then being adjusted to any new convenient height. The second celluloid scale is then set to read zero at this height of the arm.

Subsequent points are transferred in the same way, except that where the values of the horizontal coordinates differ from zero, the machine, in addition to being rotated through 180°, must also be moved over to coincide with coordinate lines which are comparable to those used for the determination of the original point. If a magnifying factor is used, the coordinate values must be multiplied or divided by this factor in taking up the new position. If, for any reason, a mirror-image copy is desired, one of the coordinates should be made a mirror image. Marking the zero lines as shown in the figure, the one which is made a mirror image should be the x coordinate, since if the x coordinate be not a mirror image, it will be found that the pointer reaches across the copy and hampers the operator.

It may occur with large models that some part of the original interferes with the rotation of the machine. In this case, the zero lines for the copy may be removed some distance from those for the original. They may even be drawn on an entirely different sheet of paper on another table. In this case, it may not be desirable to rotate the machine, thus obviating difficulties of orientation with the mirror images.

THE GROSS AND HISTOLOGICAL ANATOMY OF THE BRAIN OF A CYCLOPS

H. M. ZIMMERMAN AND KONSTANTIN LÖWENBERG[1]

SIX FIGURES

The congenital anomaly designated by the term cyclops consists of a bilateral symmetrical malformation of the head in which there is an absence of some of the median structures, or in which at least these parts are poorly developed. In more than one hundred cases of this condition that have been reported there has been sufficient individual variation to make it at once apparent that this definition of cyclops is but partly applicable in specific instances. Perhaps, as suggested by Schwalbe,[2] a more inclusive definition would be: that anomaly in which the eyes are so closely adjacent as to lie within a single orbit.

The most striking abnormality externally appears to be a single or an apparent single eye which lies in the midline. This eye, however, is most commonly produced by a fusion of two components and usually has two corneae, but there may be a single cornea. Also, there can be two separate eyes lying in a single orbit. The latter lies in a position where normally the root of the nose is present. Other structures showing some degree of malformation are the nose, the skull, and the brain. A careful histological examination of the brain in this instance has revealed a number of interesting

[1] From the Department of Pathology, Yale University School of Medicine, and the Pathologisches Institut des allgemeinen Krankenhauses St. Georg, Hamburg (Prof. Friedrich Wohlwill, prosector), through the courtesy of Doctor Weber, of Stade, Germany, who submitted the material for study.

[2] Schwalbe, E. 1913. Die Morphologie der Missbildungen des Menschen und der Tiere, Theil 3, S. 205.

findings which have previously not been reported and which justify, therefore, another report of a condition which has so ably been described by Schwalbe.

HISTORY

The mother of the cyclops was a thirty-year-old woman who had already given birth to three healthy boys. In the present instance delivery was performed by cesarean section in the eighth month of pregnancy, because of placenta previa. There was an increased amount of amniotic fluid. The child lived two hours following delivery. No other details in the clinical history were obtainable.

NECROPSY FINDINGS

Gross

The congenital malformations are confined to the head. The skull is of an approximately normal contour. The face shows the typical abnormalities of a cyclops (fig. 1). The nose (proboscis) consists of a cylindrical projection, about 3 mm. in diameter and 5 mm. in length, which ends in a flattened, circular disc about 5 mm. in diameter. No openings are found in this structure. It is present in the midline about 2 mm. above the center of the eye and projects perpendicularly forward.

A single orbital cavity is present which contains the greatly malformed eye, consisting of a fusion of two eyes with a single cornea. The cavity is rhomboid in shape, and between its lower angle and the mouth is a distance of about 1 cm.

There is a single floor common to both anterior cranial fossae (fig. 2). The crista galli is completely absent and there is also a corresponding absence of the usual depressions of the orbital plates toward the midline. The sella turcica is

Fig. 1 Head of cyclops, showing proboscis and medially placed orbital cavity containing the single malformed eye.

Fig. 2 Base of skull, showing the malformed anterior cranial fossa and the round posterior cranial fossa.

Figure 1

Figure 2

narrow and the posterior wall of the sella is rather prominent. Anteriorly, the boundary of the sella is rounded and concave, and as a consequence the anterior clinoid processes are closer together than they are normally. The middle fossae and the petrous portions of the temporal bones are normally formed. The posterior fossa is of a decidedly round form, the diameter at the base being 5.6 cm. in length and 5 cm. in width.

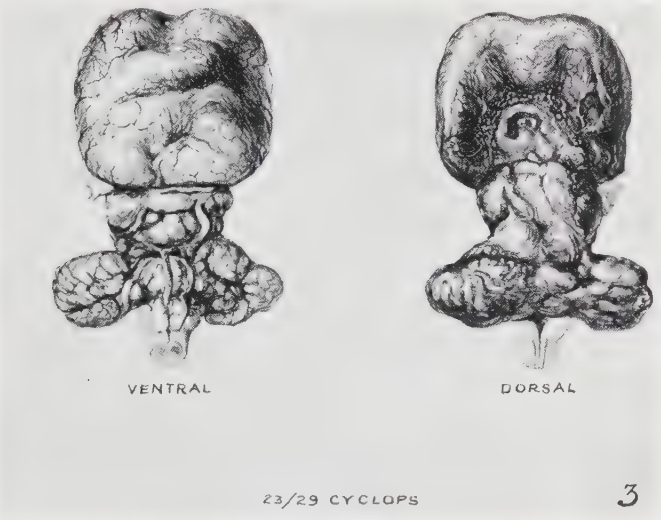
In the removal of the brain the vertebral arteries have been cut close to the basilar artery. The latter is large and well developed and normally situated. Two arteries take origin on either side from the basilar to supply the anterior and posterior parts of the inferior surfaces of the cerebellum. Two very small pontine arteries are also present bilaterally. The usual superior cerebellar arteries are present. On either side a rather prominent artery has an anomalous origin from the junction of the basilar and the posterior communicating artery, and these vessels also supply the inferior surfaces of the cerebellum. The posterior cerebral arteries are present, as are also the posterior communicating. The middle cerebral arteries are both present; the right gives rise to the anterior cerebral and the left is an end artery, in the sense that there are no anterior communicating arteries to complete the circle of Willis anteriorly. Internal carotid arteries cannot be identified.

Grossly, the only cranial nerves that can be found and identified are the fifth, sixth, seventh, and ninth, and these have a normal origin. The olfactory and optic nerves cannot be found. The optic chiasm is not present.

The greatest abnormalities lie in the pallium (fig. 3). This is represented by a somewhat circular flattened disc 3.5 cm. in diameter. It is slightly convex on the inferior surface and concave on the superior, presenting the appearance of the head of an inverted mushroom. There is no separation into cerebral hemispheres. Small elevations are present on both the superior and inferior surfaces and these represent the only evidences of cerebral convolutions. The antero-inferior surface of this disc-like structure shows a groove in the mid-

line which probably represents the undeveloped longitudinal fissure.

The midbrain as a whole appears to be well developed and corresponds in this development to the stage reached by an



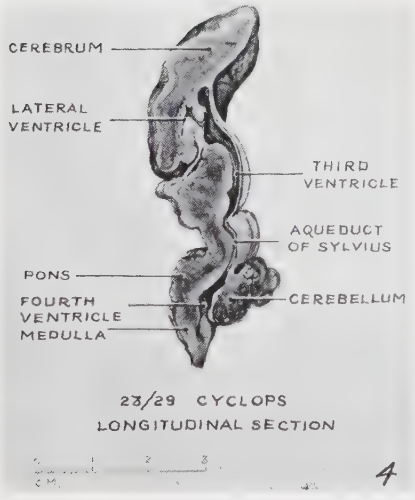


Fig. 3 Brain of cyclops.
Fig. 4 Longitudinal section of brain of cyclops.

embryo of this age. Anterior to the corpora quadrigemina the roof of the third ventricle has been ballooned out into a cyst-like structure whose wall is formed by the roof of the third ventricle and markedly thickened and adherent lepto- and pachymeninges. On the inferior surface of the midbrain can be seen the well-formed infundibulum and pituitary gland. The interpeduncular fossa is represented by a definite groove.

The pons, cerebellar lobes, and medulla are quite well developed. On the inferior surface of the medulla, just lateral to the median sulcus, can be seen single marked elevations representing the inferior olives. The fourth ventricle appears large. The median fissure and the median sulcus are represented by deep grooves.

A longitudinal section through the entire brain (fig. 4) reveals an average thickness of the cerebrum of 6 to 9 mm. The latter is a solid structure having a small intercommunicating cavity in its posterior end which represents the lateral ventricles. The cortical gray matter is fairly well demarcated from the white matter by its paler color. The inferior extremity of this central cavity is in communication with the cyst-like structure which represents the greatly dilated third ventricle and which has already been described. The posterior end of this ventricle gives rise to a well-developed aqueduct which is shaped like the letter S and is patent throughout. The thickness of the pons in its midportion is 3 mm. and its length is 9 mm. The cerebellar folia are well recognizable. The greatest length of the fourth ventricle is 13 mm. In its anterior portion the roof of this ventricle is made up of the intact velum and the cerebellum. The posterior end of this cavity is left open by reason of the fact that the cerebellum fails to extend down to the medulla.

The pallium and midbrain are joined by a narrow pedicle (cerebral peduncle) which measures 3 mm. in thickness and which has a deep furrow on the ventral surface, partially separating these two structures.

Microscopic

Beneath the pia of the cerebrum (fig. 5) is present a narrow band of densely placed and dark-staining small round cells, three or four in thickness. Beneath these cells is present a

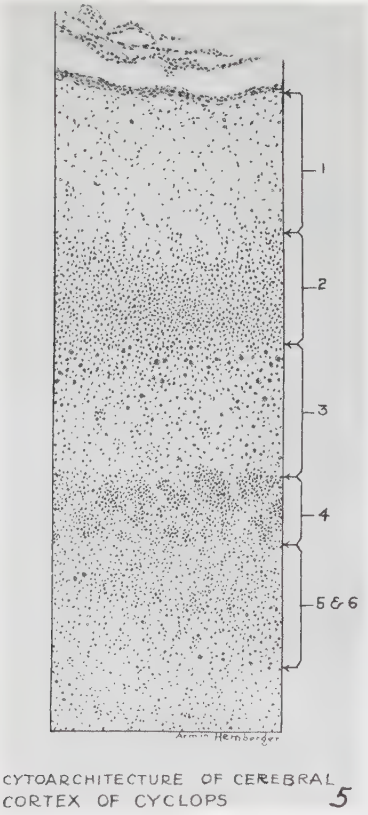


Fig. 5 Drawing of cerebral cortex of cyclops, to show cyto-architectural structure. Note distinct layers. Delafield's hematoxylin without counterstain. × 65.

wide, pale, and acellular zone which forms the molecular layer. Next can be seen a fairly continuous band of small, round, dark-staining cells which corresponds to the external granular layer. The third layer contains relatively few cells, which are of a larger size than those of the second layer, these cells

being more numerous in the zone adjacent to the second and less numerous near the fourth layer. These cells have ample cytoplasm and large pale nuclei, but they are still round in shape. The fourth or inner granular layer is composed of small round cells similar to those forming the second layer, only these cells do not form a continuous band as do the cells of the second layer. The fourth layer has an appearance similar to the glomerular zone in the hippocampal cortex. The next, or fifth, layer is composed of a mixture of these

Fig. 6 Photomicrograph of cerebellum of cyclops. Delafield's hematoxylin without counterstain. × 58.

same small round cells and the neuroblasts of the third layer, with the smaller cells predominating. This last layer, together with the sixth, forms a band of cells which is rather wider than any of the other layers and gradually fuses with the subcortical white matter. The entire cortical cellular arrangement corresponds to this description, with a few minor and unessential variations in different parts of the cortex.

In the cerebellum (fig. 6), immediately beneath the pia, is a band of small, dark, round cells. Then follows an acellular band corresponding to the molecular layer of more mature

brains, and this is followed by an inner granular layer of small round cells. In the zone of transition between the molecular and the granular layers are present large cells which resemble somewhat the Purkinje cells. The cores of white matter projecting into the granular zones are primitive in their development.

In the regions of the brain corresponding to the central gray matter are found large cells with abundant cytoplasm which represent the primitive ganglion cells of these zones. However, the normal cellular architecture of the central gray matter is still lacking in development. In the pons and medulla are also found nests of ganglion cells representing the anlagen of the nuclear masses found in normal brains, but the apparent lack of orderly arrangement of these groups of cells makes it quite impossible to identify any of them.

The ventricular ependyma is well formed and is composed of tall columnar cells. The leptomeninges show no abnormalities.

COMMENT

In common with the many cases collected by Schwalbe, the case presented here has a single orbital cavity in which is present one eyeball with one cornea. This represents the usual finding in cyclops, though the eye may have so many variations as to make a single description invalid for all cases.

The nose or proboscis in this monster is a small structure placed in the midline above the eye—a condition found in the majority of instances. The proboscis may be a double organ, however, or one having a diameter of a centimeter or two. There is a complete absence of the nasal bones and a malformation of the ethmoid bone, with flattening of the anterior cranial fossa. The sella turcica is slightly malformed. These are rather constant findings in all the reported cases.

The cerebral malformations are limited in the majority of instances to the forebrain and the midbrain. The mesencephalon, metencephalon, and myelencephalon may be normal. The forebrain is never divided into hemispheres, and consists of a single mass that is of horseshoe shape with the

concavity upward and posteriorly. It contains an undivided cavity which corresponds to the two lateral ventricles. The corpus callosum, septum pellucidum, and fornix are absent. The rhinencephalon is almost constantly absent. Only rarely is there present a single medially placed olfactory nerve, of rudimentary development, representing a fusion of the two olfactory nerves. If the corpora striata are at all present, they are poorly developed. The thalami optici are often fused into a single structure. The base of the diencephalon lacks any differentiation. In place of the corpora mamillaria, infundibulum, and the substantia perforata there is present a pear-shaped mass whose apex gives rise to a single optic nerve. Occasionally two definite nerve bundles form an optic chiasm that gives rise to two separate optic nerves. At times the optic nerves are absent.

A rather constant and unusual finding is present in the roof of the mesencephalon. Normally, it is a thin structure which is reflected over and covers the choroid plexuses. In cyclops this roof is ballooned out to form a vesicle which at times reaches an extraordinary size and can, indeed, cover most of the forebrain and fill the greater part of the cranial cavity. Its wall is quite thin and not infrequently is united with the dura, occasionally being torn in the removal of the brain. The epiphysis and hypophysis are as often absent as they are present. The cranial nerves from the third to the twelfth are usually present. Changes in the cord when they occur present no uniform picture.

The vesicle usually produced by an outpouching of the roof of the mesencephalon, in the present instance, is produced by a similar outpouching of the roof of the diencephalon. Indeed, this vesicle simply represents a dilated third ventricle. The cerebral aqueduct appears quite normal.

In none of the cases reported hitherto has any adequate description been given of the histological structure of the brain. It is a matter of some interest, therefore, to find in a brain which grossly presents so many anomalies a rather normal, if immature, cyto-architectural structure in the cortex.

There is a definite arrangement of the cortical neuronal elements into six layers, and although the different cortical areas lack the usual differentiation, it is nevertheless surprising to find the fundamental and characteristic six-layer arrangement so sharply defined. To be sure, the individual ganglion cells are not as maturely developed as is to be expected in an eight-month fetus. On the other hand, the histologic picture of the cerebellum is quite in keeping with that of a normal fetus of the same age. The architectural disposition of the ganglion cells forming the central gray matter as well as of those forming the nuclear structures in the pons and medulla is of such a nature as to make impossible the positive identification of the structures involved.

SUMMARY

The cyclopic monster was the product of the third pregnancy in a normal woman of thirty years and was delivered by cesarean section in the eighth month because of placenta previa. The child lived two hours. It presented the usual malformation of the head, consisting of a single orbital cavity placed in the midline and containing one eyeball with one cornea. A rudimentary proboscis occurred in the midline above the eye. The skull was malformed to the extent that there was but a single flat anterior cranial fossa produced by a maldeveloped ethmoid bone. The brain presented the usual horseshoe-shaped, unpaired prosencephalon and a greatly dilated third ventricle. The olfactory and the optic nerves, as well as the optic chiasm, were not present. Positively identified were only the fifth, sixth, seventh, and ninth cranial nerves. Histologically, the cerebral cortex was the normal six-layer type, though the individual ganglion cells were immature in their development. The cellular architecture of the cerebellar cortex was that of a normal fetus of eight months. The basal ganglia and the nuclear masses of the pons and medulla were poorly developed.

NEW DIAGRAMS ILLUSTRATING THE TRANSFORMATION PERIOD OF THE HUMAN SPERMATID, AS PREPARED BY THE LATE PROFESSOR BOWEN[1]

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ELEVEN FIGURES

For some years the writer has been dissatisfied with the diagrams used currently in texts to illustrate the important stages occupied by the transformation of the human spermatid into a mature spermatozoon. Search of the literature having failed to reveal any figures of direct teaching use, the attempt was made to construct such anew. It soon became apparent, however, that the intricacies, contradictions, and factual lacunae presented a combination not to be successfully surmounted except by one intimately familiar with this specialized field. Accordingly, I turned for aid to my friend from undergraduate days, Prof. Robert H. Bowen, then of Columbia University. His qualifications and experience in this field are too favorably known for any specific comment, while his contributions within a relatively few years to the permanent literature will render his name enduring. To my request that he draw up a few rough diagrams including the specific points that had puzzled me he replied that he would gladly do so. In due time there arrived a parcel containing, instead of the hasty working sketches I had anticipated, a series of beautifully drawn and labeled stages ready for publication, together with some miscellaneous memoranda concerning them. This was a most characteristic act of the true and generous friend Professor Bowen ever was, and now

[1] Contribution no. 154.

that his sudden death has removed from cytology the one who was perhaps destined to be her foremost American exponent, I am glad to make available in full form this unfinished but useful cytological fragment. The reader should realize that the citations to be given are taken directly from personal correspondence, and in justice to Professor Bowen, it must be emphasized that our long intimacy had made these interchanges of the most informal and unedited type.

In his letter of June 28, 1928, accompanying the diagrams, the following statement occurs:

> I have been so busy here and there that the diagrams of the human sperm have necessarily gone slowly. It turned out to be a very much longer job than I had anticipated, and so I have been that much the longer in getting it done. I finally decided to work out a set of figures as carefully as I could, and these are enclosed herewith. They are drawn as nearly to scale as the data available would permit. I am so disgusted with the frightful figures of human sperm formation so universal in medical texts, that I spent rather more care than otherwise in the production of a series that might be more acceptable. Much of it is of course added from our knowledge of other mammals, but this seems justifiable, and in any case the errors are nothing to compare with the ones current in present figures. The problem of the centrioles is the most difficult, and I have done with them what I could. When the matter has been more thoroughly investigated, the corrections can be made, but this is not apt to happen very quickly. I am sending several sheets of notes by way of explanation of what I have done, and of answer to your questions. I hope the whole thing will be understandable, but I have had to put on the final touches under great pressure from other sources, and I may have made some slip or omission. In case there is anything wrong or not clear, you can write me.
>
> I have only one request to make. So far as I am concerned you may, if you like, use the drawings just as I send them. If you prefer to reconstruct them or throw them out the window, please feel very free to do so. But in case they can be made immediately available, may I merely make the request that they be ultimately returned to me after the printer is through with them. There is of course no hurry. If it is a matter of years, it will be all right.

It is scarcely necessary to add that the series of diagrams was used without change, but the notations, valuable as they

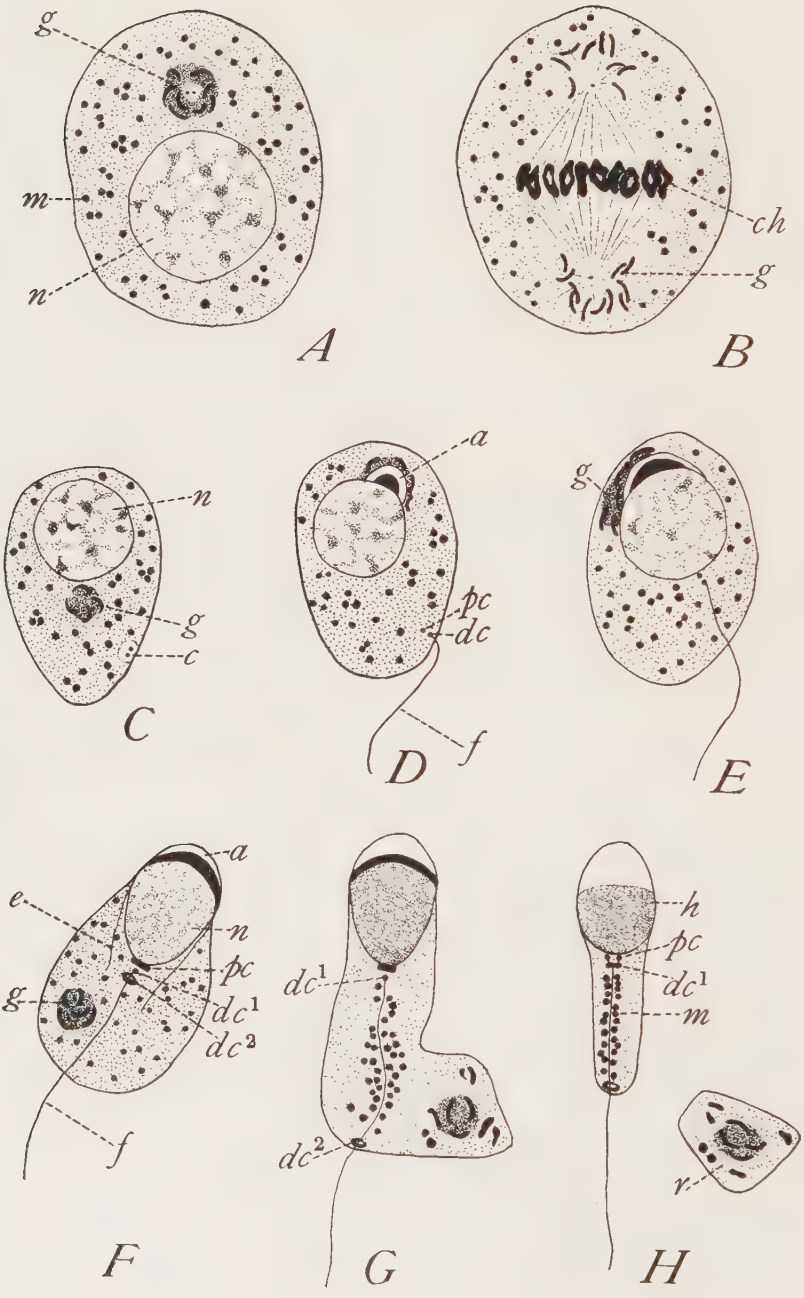
are, could find but small place in a student text and so with few exceptions had to be omitted. However, I had promptly written, urging publication, and that the presentation of this material in some form was definitely contemplated seems reasonably clear—as the last paragraph quoted also vaguely intimates. These facts and the inherent importance of making the data available as a part of the permanent scientific record have enjoined the writer to see that publication was accomplished. The drawings with their legends and accompanying notes should be self-explanatory, and for this reason the remaining paragraphs are transcribed directly from the original memoranda furnished by Doctor Bowen.

Aside from the too early expulsion of the protoplasmic remnant, the figures are simplified in three respects:

1. Most mammalian sperms begin the formation of proacrosomal vesicles and granules during the primary spermatocyte stage. Many other kinds of sperms do not. The latter condition is much the simpler, and since the facts are not definitely known in man, I have taken the liberty of simplifying the figures to that extent. The probable formation of part of the acrosomal material within the idiosome during the growth period should probably be pointed out.

2. According to many workers, the sperm of some mammals has a slight dilatation in the region of the middle piece. Gatenby and Woodger have shown this to be probably a piece of the Golgi complex left behind when the remainder is cast out in the protoplasmic remnant. The conditions in man are not definitely known, and I have omitted all Golgi material from the later figures (I to K). The possible, or even probable, retention of a similar fragment of this material ought perhaps to be noted.

3. The structural features of the acrosome have been purposely, and I think justifiably, simplified so that only the acrosomal vesicle and its granule are shown. Comment seems to me unnecessary.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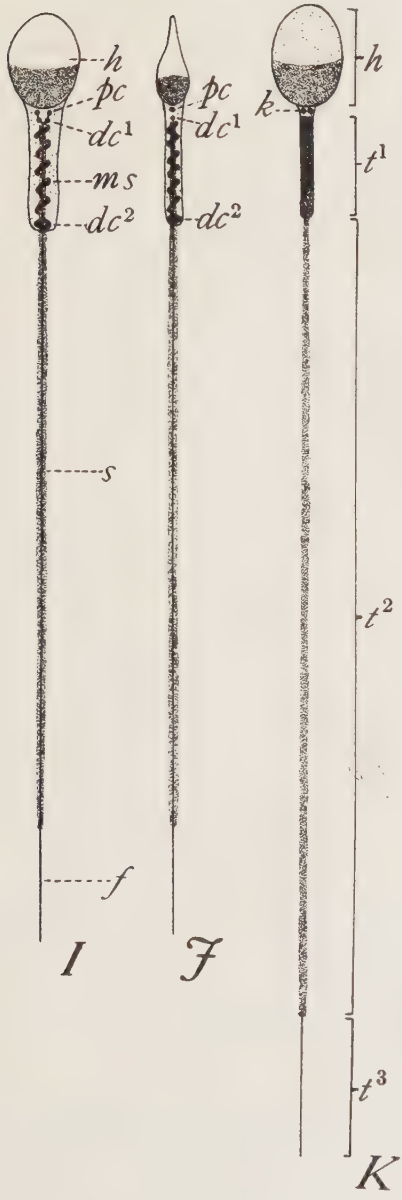

EXPLANATION OF FIGURES

Semidiagrammatic stages illustrating the development of a human spermatozoon. × 3300. Based on the work of Meves, Broman, Retzius, and Branca on the human sperm, and of Meves, Duesberg, and Gatenby and Woodger on the sperm of the guinea-pig. (Reproduced from Arey, "Developmental Anatomy," 2nd edition, by permission of W. B. Saunders Co., publishers.) A, Primary spermatocyte during late growth period; B, metaphase of first spermatocyte division; C, spermatid immediately after the conclusion of the second spermatocyte division; D to H, earlier stages in the transformation of the spermatid into a sperm; I (plane view) and J (profile view), late stage in the formation of a mature sperm; K, mature sperm (after Retzius) as seen in plane view. *a*, acrosome; *ch*, chromosomes; *c*, centrioles; *dc*, distal centriole; dc^1, granular derivative, and dc^2, ring derivative, of the distal centriole; *e*, 'manchette'; *f*, tail or axial filament; *g*, Golgi apparatus plus idiosome (idiosomic substance represented by stipple); *h*, sperm head (nucleus plus acrosome); *k*, neck region; *m*, mitochondria; *ms*, mitochondrial spiral; *n*, nucleus; *pc*, proximal centriole; *r*, protoplasmic remnant (here represented for the sake of clearness as being cast off at a somewhat too early stage—the actual separation of the remnant probably takes place in a stage more nearly like that represented in I and J); *s*, sheath of the tail filament; t^1, middle piece, t^2, principal piece, and t^3, end piece—subdivisions of the tail.

In figure B the idiosomic substance is omitted because its whereabouts are not certainly known. Presumably it accompanies the Golgi batonettes, but is invisible because of inadequate technique. The chromosomes are put in roughly from some of Painter's figures.

In figures G and H attention should be called to the general nature of the cast-off protoplasmic remnant (bulk of the Golgi apparatus, some undifferentiated cytoplasm, and general detritus of sperm formation). These balls of protoplasmic débris are ultimately ingested by the Sertoli cells and are not, therefore, a part of the ultimate seminal fluid.

In figures H to K the exact shape and extent of the acrosome are unknown. Probably it fits over the sperm nucleus more or less like a cap, but this need not be very specifically mentioned. See Branca, '09.

The centrioles occupy a place within the Golgi complex during the primary spermatocyte stage, but in the spermatid no such topographical relation ever exists. This is as definite as anything can be in cytology. I strongly advise against the term centrosome, widely and always incorrectly used in America. Better speak of the centrioles or central bodies—which is all that is here required. A good term for referring to the centriole arrangement as a whole is 'central apparatus' —but this refinement seems scarcely necessary for general purposes. The most difficult part is the history of the centrioles subsequent to figure G. Up to this point accounts agree with each other and also with the conditions in other mammals. Beyond this point their behavior is pretty much guesswork. I have omitted in figures F and G the peculiar little extensions sometimes shown attached to the proximal centriole—and other irregularities which seem to me too trivial for consideration in a diagram for the present purpose.

The division of the proximal centriole (*pc*) as shown in figures H, I, and K is probably a frequent, if not invariable, occurrence, as indicated by the accounts of Retzius, Broman, and Branca. Retzius shows the divided granule even in preparations of completed sperm (fig. K). That these

granules are probably the descendants of the proximal centriole should be noted in the text—they lie in the so-called neck region of some authors. A very bad name, the neck, but it has got a pretty firm hold in accounts of mammalian sperms. Wilson's statement to which you refer is probably derived more from Meves' account of early stages than anything he actually says about the mature condition—although Meves does give a diagrammatic figure of a mature human sperm showing an undivided proximal centriole. The evidence that it is divided seems to me, however, fairly clear, though not perhaps decisive.

The connection of the proximal and distal derivatives in the neck region by delicate fibers is noted at times by Branca and Broman, and may very likely occur—as in the case of some other mammalian sperms. It is indicated in figure I, omitted in figure J. This is not a very important matter in any event.

The final history of the proximal part (dc^1) of the distal centriole is quite uncertain. Apparently Meves did not think it divided in man, but it is known to do so in some mammals, and may do so sometimes or regularly in man. The undivided condition (dc^1) is shown in figure H, the divided condition in figure I. Figure J, being in profile, would show only one in any case. Branca, also, seems to believe that the undivided condition exists, but Broman and Retzius incline to the other view (at least sometimes). It is a case of pay your money and take your choice; the undivided condition would be the simpler one, but the evidence is equally good that the divided condition occurs sometimes. If the divided condition does occur, the manner of insertion of the tail filament is unknown. I have cut this Gordian knot the best I could (fig. I), and the less said about it the better. The statement that the conditions in man are as described by Meves in the rat, made, as you note, in Wilson's book (p. 378), is rather ambiguous. Certainly Meves did not so describe things. I would disregard it and take the facts as known, considering Wilson's statement as a slip of the pen.

My representation of the Golgi rodlets plus idiosome is the one usually given, particularly following Gatenby. I think it is probably an inadequate picture of the true situation and that ten years from now in another revision you would want to correct it. But for the present I think it best to follow the current description, especially as it makes very little matter for the present purpose.

The granular mitochondria are taken over from Duesberg on Cavia, but Branca gives faint indications of a similar condition in man, and I think it the only safe representation for the present.

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LEFT OBLIQUE INGUINAL HERNIA IN A MALE DOG

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THREE FIGURES

Inguinal hernias are a great rarity in male dogs. Having observed recently such a case, it appeared to us worthy of record.

In an adult male dog, weighing 12 kilograms, the presence of an easily reducible left inguinal hernia was noted while the animal was prepared for a cholecystectomy. The photographs illustrating the condition were obtained at the close of the experiment about three weeks following the operation.

Figure 1 shows the enlargement of the left half of the scrotum to about three times its usual size. A slight prominence of the skin is noted over the external orifice of the left inguinal canal and along the course of the spermatic cord.

In figure 2 the hernial sac is exposed by removal of the skin and fasciae. It shows the relative size of the contents of the scrotal sacs on each side.

Figure 3 shows the hernial sac opened and its content exposed. The loop of bowel was identified as the lower ileum and measured about 15 cm. in length.

COMMENT

Surgical repair of inguinal hernias in three female dogs had been reported by Beall in 1905(1). He quotes Parascandle's report as being the only one of an inguinal hernia in a male

[1] This study has been conducted under the joint auspices of the Douglas Smith Foundation for Medical Research of The University of Chicago and the Otho S. A. Sprague Memorial Institute.

Figure 1

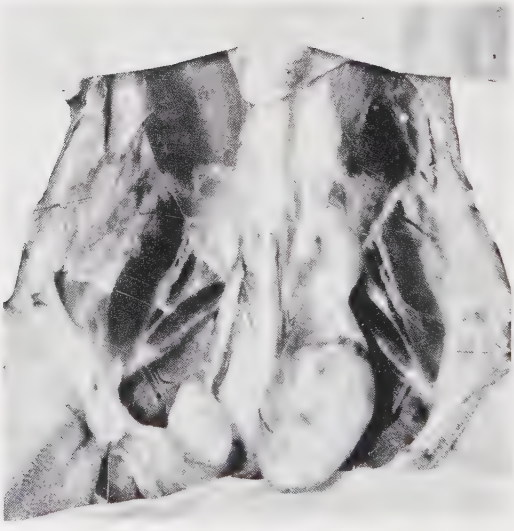

Figure 2

dog that he could find in the veterinarian literature. Data on hernias of dogs are relatively scarce and we could find no record of a case similar to ours.

Warwick's(2) extensive studies on hernias in swine brought out the interesting fact that 1.68 per cent of the male pigs had inguinal and 0.60 per cent had umbilical hernias. Among the female pigs of the same herds, 1.16 per cent had hernias, practically all of which were umbilical, although a few had

Figure 3

inguinal hernias. Warwick(3) explains this in the following way: In swine the female normally does not possess an open inguinal canal, and so inguinal hernia in this sex is rare. In some species of animals, as the dog, the inguinal canal in the female contains the round ligament of the uterus, and thus always remains open. According to this author, inguinal hernia of the female is quite common in species having this anatomic arrangement.

Sutton(4) has called attention to the fact that the connection between the cavity of the tunica vaginalis propria testis

and the peritoneal cavity remains open in the majority of the mammals, except perhaps the chimpanzee and gorilla. Yet he noticed the presence of an inguinal hernia in only three out of more than 800 monkeys which he examined postmortem. The series included "examples of all the chief species, excepting the gorilla."

These data and the facts known of the dog and of man are of much interest. In the adult male swine and dog the cavity of the tunica vaginalis propria testis communicates with the peritoneal cavity as in other mammals; in man it does not. Inguinal hernias are frequent in male, and scarce in female, swine. In male dogs inguinal hernia is almost unknown. Its frequency in the female is, according to Beall(1), in part at least, due to the weakening effects of pregnancy on the abdominal wall. In human beings the sex occurrence of inguinal hernias is similar to that in the swine, i.e., the occurrence is more frequent in the male. All these facts suggest that heredity (anatomic conditions) and mechanical causes play perhaps an equal part in the etiology of inguinal hernias.

SUMMARY

A left oblique inguinal hernia in a male dog is described. From a comparison as to frequency of inguinal hernias in the dog, swine, and man, the writers conclude that heredity (anatomic conditions) and mechanical causes play perhaps an equal part in the eitology of inguinal hernias.

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THE ORIGIN OF THE BLOOD OF THE 'PLACENTAL SIGN'

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THREE PLATES (NINE FIGURES)

Uterine bleeding during the period of pregnancy is a phenomenon which has been observed in several species of animals. Long and Evans ('20) noted the presence of blood in the vagina in pregnant rats from the thirteenth to the sixteenth day of gestation and they designated this bleeding as the 'placental sign.' Subsequently, Hartman ('28, '29) noticed a similar 'placental sign' in Macacus rhesus. In thirty-two macaques he followed the duration of the uterine bleeding by vaginal examinations and determined that the presence of blood in the vagina may be regarded as an early indication of pregnancy in that species.

It has been known for years that in certain animals extravasations of blood occur into the uterine cavity during gestation. These extravasations have their source in the uterine walls underneath or in the neighborhood of the placenta. Thus in many rodents the presence of maternal blood free in the uterine cavity has been observed; and in carnivores as well, the extravasates are more localized and are designated as the border or central haematomata so characteristic of these animals. Indeed, it has been recognized that such extravasates form a readily accessible store of iron compounds which becomes available to the fetus to meet its needs for the production of hemoglobin.

The observation that uterine bleeding may manifest itself as the 'placental sign' in some animals by the escape of visible

blood into the vagina is an interesting corollary. It needs, however, to be fully ascertained whether the extravasations of blood long known to occur in the pregnant uterus are the source of such external bleeding. Moreover, more information is required concerning the time and manner of occurrence of the extravasates themselves around and beneath the placenta. Practically no effort has been made in the past to trace by adequate means, especially by injection methods, the mode of escape of the extravasates from the uterine vessels.

Goldmann ('12) is the only investigator who has actually attempted to determine the anatomical pathways involved in the production of uterine extravasates in pregnant animals. In several gravid rats, by injection of India ink into the uterine arteries, he demonstrated the anatomical pathway for the escape of blood from the vessels of the endometrium into the uterine cavity. He described vessels in the endometrium pursuing a course from the mesometrial surface of the uterus toward the antimesometrial surface. These vessels give off, toward the surface of the endometrium, fine branches which anastomose in a plexiform manner. Certain of these twigs ultimately pierce the cuboidal layer of epithelial cells clothing the endometrium in the antimesometrial portion of the uterus, so that the vessels communicate freely with the uterine cavity. Goldmann believed that the openings of these vessels possess a valve-like action and that the amount of blood escaping from them in life under physiological conditions is regulated by the rate at which the blood is assimilated by the wall of the yolk sac.

Finally, to shed more light upon the relationship of the external bleeding of pregnancy to the internal presence of hemorrhagic extravasates, Wislocki and Hartman ('29) studied carefully the entire uterus of a pregnant macaque. In this macaque, killed one month after conception and on the tenth or eleventh day of the bleeding, they traced the external bleeding to its source in the endometrium. In the latter, blood vessels were seen to have ruptured into the

lumina of many of the uterine glands, from which blood was observed to escape freely into the lumen of the uterus, whence it found its way into the vagina.

The present investigation was undertaken to extend our knowledge in the common laboratory animals of the possibility and manner of the escape of blood into the uterus during pregnancy. These observations also were planned to aid in elucidating the possibility of external bleeding and its relationship to internal bleeding. It was decided to study the question of uterine bleeding by means of vascular injections of gravid uteri.

METHOD AND MATERIAL

By means of vascular injections it was thought that it would be possible to trace the pathways of escape of blood from the endometrium into the uterine cavity. For the purpose India ink was selected as the most useful injection fluid.

The technique was simple. The animals were etherized and bled to death through an incision in the right ventricle. The descending aorta was exposed and cannulated. Upon injection, as soon as the ink flowed freely from the right ventricle, the inferior vena cava was ligated. Sufficient pressure was used to insure complete injection of the uterus, as judged by inspection of the uterine circulation. At no time, however, was enough pressure employed to produce visible rupturing of the uterine vessels. After completion of the injection, the entire reproductive tract was removed and fixed in Bouin's fluid. Subsequently, blocks of tissue were embedded in celloidin, sectioned, and variously stained.

A series of pregnant rats received injections. Rats, followed by the vaginal-smear method, were mated and killed on known dates after fertilization. Smaller series of guinea-pigs and rabbits of known dates of coition were also used. A few pregnant cats and dogs of unknown dates of coition received similar injections.

OBSERVATIONS

The rat material affords the most complete series for study. In order to discuss uterine bleeding in this animal, it is of value to describe the blood supply to the endometrium at the different stages of gestation.

In the non-gravid uterus at the time of ovulation there are numerous vessels of moderate size visible in the deeper portion of the mucosa, while the superficial portion of the mucosa is relatively avascular. After ovulation, during the period of fertilization and the initial stages of implantation, covering about a week's time, the endometrium undergoes perceptible changes, consisting of a pronounced hypertrophy of the uterine glands and of a gradually increasing vascularization of the mucosa. The blood vessels of the deeper layer of the endometrium increase in both size and number. The outer or superficial region of the mucosa also becomes increasingly more vascular, although remaining, as before, relatively less vascular than the deeper portion.

Beginning toward the end of the first week of gestation and continuing through the second week, the vascularity of the endometrium increases tremendously. This augmentation of the blood supply is due to the ingrowth of a plexus of fairly large vessels into the outer zone of the mucosa. This plexus comes ultimately into intimate contact with the uterine epithelium, which in many places is all that separates the vessels from the uterine lumen. Pari passu with the development of this plexus, the deeper layer of the endometrium appears to become cellularly more compact and its vessels fewer in number and size. The vascularization of the mucosa reaches its height about the twelfth to the fifteenth day of the period of gestation.

During the third week of pregnancy, there commences a gradual decrease in the vascularity of the entire mucosa, so that near term, on the twentieth day of gestation, the mucosa has become much narrower, cellularly more compact, and relatively avascular. The plexus in the outermost zone of the mucosa dwindles rapidly, leaving the endometrium with

fewer blood vessels than at any previous period. These cyclical changes in the endometrium during gestation are illustrated in figures 1 to 6.

After employment of the customary technique, free ink was found in the uterine lumen of rats pregnant from twelve to fifteen days. This period coincides with the stage of the greatest vascularity of the mucosa. It is interesting that Long and Evans detected vaginal bleeding, the so-called 'placental sign,' from the thirteenth to the sixteenth day.

The origin of the extravasated blood occurring in the uterus from the twelfth to the fifteenth day in the pregnant rat could be determined. At this period the placenta is well established in the mesometrial half of the uterus. At the antimesometrial pole of the blastocyst, the uterine lumen has been reestablished and a decidua capsularis encloses the antimesometrial surface of the blastocyst. The decidua capsularis, together with the outer wall of the blastocyst (Reichert's membrane) and the parietal endoderm of the yolk sac, is undergoing the initial stages of degeneration. It is while these changes are proceeding that extravasation of blood is found in the uterus. In the injected material the extravasates were traced to the rich endometrial plexus of blood vessels occupying the angle of the uterus at the line of reflection of the capsularis. This region at the same time constitutes the tissue at the paraplacental border. Here greatly dilated plexiform vessels may be seen lying immediately subjacent to the uterine epithelium. In places the walls of these vessels have ruptured, destroying the delicate layer of cuboidal epithelium separating them from the uterine lumen, and blood has escaped freely into the uterine cavity (fig. 7). This extravasated blood escapes in part from the uterus into the vagina, to appear as the 'placental sign.' This is possible, since by the eleventh or twelfth day the uterine lumen has been completely reestablished and communicates freely with the vagina. That portion of the extravasated blood which does not escape into the vagina disintegrates and is resorbed by the visceral layer of the yolk sac. By the fourteenth day, the capsularis has dis-

appeared sufficiently for the yolk sac to flank the uterine cavity. In assuming this topographical relationship to the uterine lumen, the yolk sac, especially toward the placental border, develops numerous vascularized folds, clothed by columnar epithelial cells which are actively phagocytic. These cells, by direct absorption and by phagocytosis, remove much of the extravasated blood, other detritus and secretion from the lumen of the uterus.

The period of extravasation of blood from the uterine mucosa is temporally circumscribed. By the sixteenth day there are no further hemorrhages. Meanwhile the capsularis and the parietal layers of the fetal membranes have disintegrated to a line close to the placental surface. During the following days the mucosa bordering the uterine cavity becomes less vascular and the uterine epithelium, where it had previously been broken by the rupture of blood vessels, undergoes complete restoration.

We have seen similar cyclical changes, although not quite so well marked, in the gravid uteri of both the rabbit and the guinea-pig.

In the guinea-pig no trace of free ink has been found in the uterine lumen at any stage of pregnancy. Neither has any erosion of the vascular walls nor discontinuity of the uterine epithelium been observed.

On the other hand, in the rabbit there is free ink in the uterine lumen in a specimen from the eleventh day of gestation (fig. 8). Dilated spaces in the connective tissue of the endometrium, containing blood and ink, communicate with the cavity of the uterus. These spaces appear to have been filled by rupture of adjacent vessels. The area involved is essentially the same as in the rat, namely, the paraplacental region. In the rabbit, however, at this stage, the uterine cavity tends to undercut the placental border, producing a deep recess beneath the placenta. In the uterine mucosa in this angle the vessels are dilated and many of them ruptured, so that ink has escaped into the uterine lumen through gaps in the overlying uterine epithelium.

In the dog and cat, which in their placental arrangements differ very much from the rodents, ink has escaped, in our injections, into the uterine lumen from a very specialized region of the endometrium. No ink was observed in the general uterine cavity, but only in those special regions of the uterine lumen associated with the presence of the border hematomas—the so-called green and brown borders of the placenta of the dog and cat, respectively. In our injections ink was found mixed with the extravasated blood of the hematomas (fig. 9). The injection reached the hematomas through blood vessels situated in the subjacent endometrium. Here at the borders of the zonary placenta, as at the paraplacental borders of rodents, the endometrium appears to be supplied with a rich plexus of dilated vessels. Some of these rupture, allowing their contents to flow into the hematomas. The extravasations do not extend far beyond the placental margins, between the membranous chorion and the endometrium, but remain localized to the confines of the curiously modified, villous chorion of the paraplacental borders proper. It appears from these observations that the extravasations of blood into the uterine cavity encountered in carnivores remain localized and cannot therefore produce a 'placental sign' by escaping into the vagina. In further support of this belief one could adduce the well-known fact that in carnivores the annular placentae divide the uterine cornua into separate uterine compartments, so that at best vaginal bleeding could occur only from the lower border of each of the most caudally placed placentae.

DISCUSSION AND SUMMARY

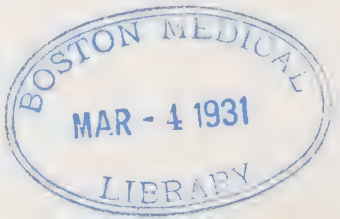
The question may justly be raised as to whether the ink seen in the uterine cavity in these experiments is a true representation of physiological anatomical pathways or is an artifact. The pictures encountered in the present specimens appear on the whole to simulate actual conditions. Two considerations lend support to this belief: first, the injection pressure used was not sufficient to cause any extravasation

of ink into the uterine cavity other than at the locations and times described; and secondly, if the extravasations of ink as they have been encountered are artificial, they portray, nevertheless, loci, which are constant and represent points of structural weakness in the uterine wall. Moreover, these localities coincide with the sites of physiological extravasations of normal occurrence.

Extravasations of blood beneath and at the borders of the placenta are of almost universal occurrence in mammals, excepting possibly in some of the placentae of the diffuse or epitheliochorial type. The actual manner of communication of vessels in the endometrium with the lumen of the uterus has been demonstrated in the present experiments in the dog and cat and in the rat and rabbit. For the time being, we have been unable to demonstrate direct anatomical communications, in the guinea-pig, between the endometrial vessels and the uterine lumen.

Actual escape of intra-uterine extravasates into the vagina, constituting a 'placental sign,' appears to be more restricted than the incidence of intra-uterine hemorrhage itself. In the rat and macaque vaginal bleeding has been amply demonstrated by Long and Evans and by Hartman. From the present observations its occurrence in the rabbit seems more than likely, but in the guinea-pig, on the other hand, our observations point to the absence of both intra-uterine and vaginal bleeding. Moreover, in carnivores, such as the dog and cat, the 'placental sign' would appear, from the present indirect anatomical evidence, to be lacking.

In the rat the 'placental sign' has been correlated with the cyclical changes in the endometrium during pregnancy. The 'placental sign' coincides with a period (twelfth to fifteenth day) during which demonstrable hemorrhage occurs into the uterine cavity. The 'placental sign' is a corollary of this hemorrhage. Neither the 'sign' nor intra-uterine bleeding occurs at any other time. In both the rat and macaque the 'placental sign' affords indubitable evidence of pregnancy.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DESCRIPTION OF PLATES

Figures 1 to 9 are photomicrographs from actual fields magnified about seventy-five diameters. The blood vessels have in each instance been injected with India ink.

PLATE 1

EXPLANATION OF FIGURES

1 Wall of non-gravid rat uterus.
2 Rat uterus, pregnant six days.
3 Rat uterus, pregnant eleven days.

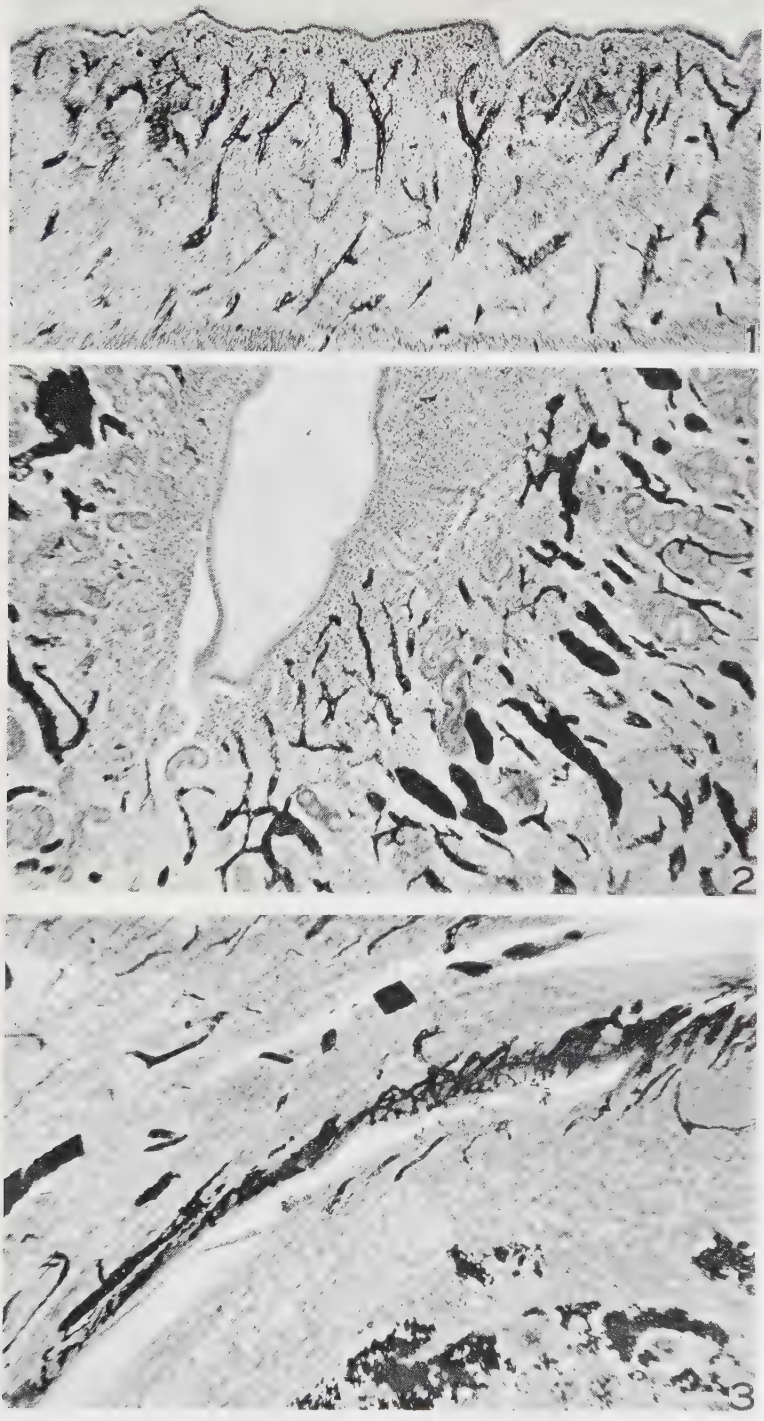
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PLATE 2

EXPLANATION OF FIGURES

4 Rat uterus, pregnant fourteen days.
5 Rat uterus, pregnant seventeen days.
6 Rat uterus, pregnant twenty days.

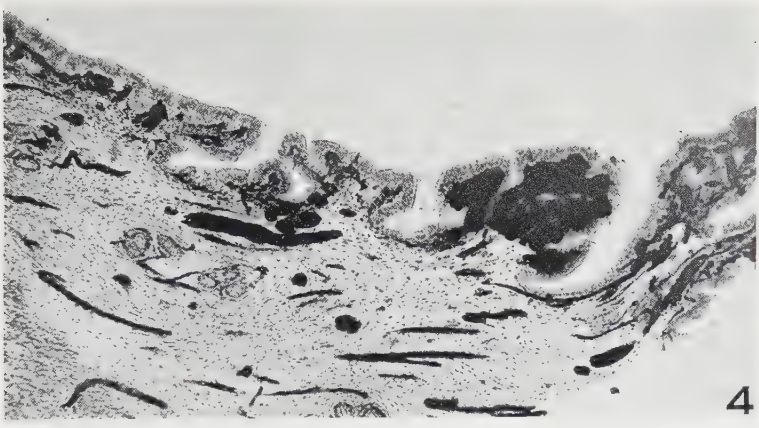
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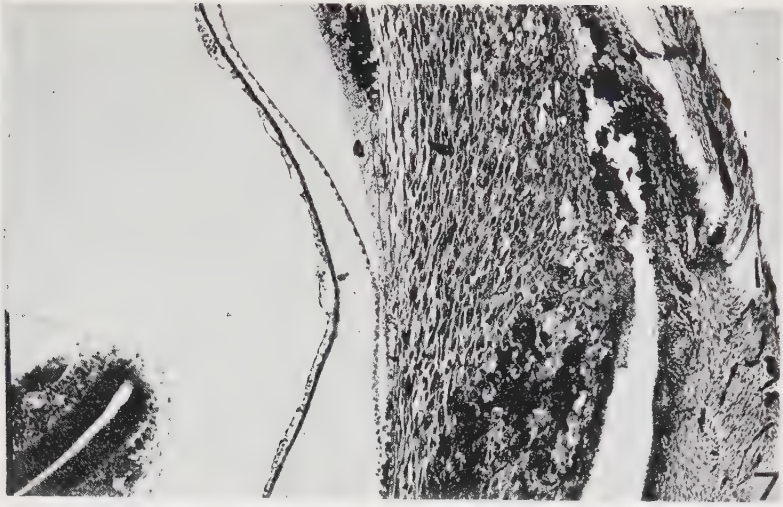
PLATE 3

EXPLANATION OF FIGURES

7 Rat uterus, pregnant twelve days. Note the escape of ink in the region of the paraplacental border at this stage.

8 Rabbit uterus, pregnant eleven days. Note the presence of ink in the uterine lumen. The extravasation has occurred from endometrial blood vessels situated at the paraplacental border.

9 Border hematoma of dog. Observe the ink which has escaped into the hematoma from adjacent endometrial blood vessels.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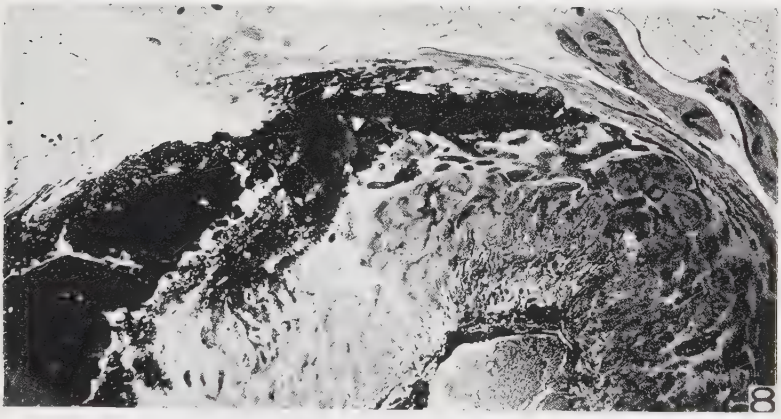
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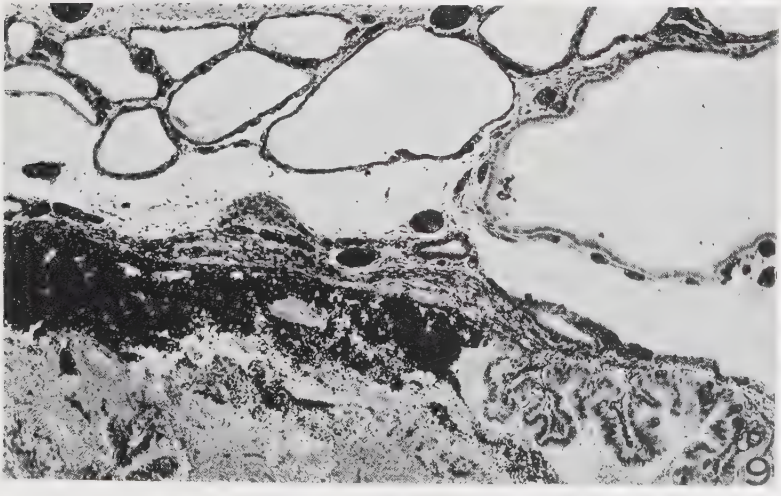
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A VOLUMETRIC STUDY OF GROWTH AND CELL DIVISION IN TWO TYPES OF EPITHELIUM,—THE LONGITUDINALLY PRISMATIC EPIDERMAL CELLS OF TRADESCANTIA AND THE RADIALLY PRISMATIC EPIDERMAL CELLS OF CUCUMIS

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THIRTEEN FIGURES

"Histology—content, like anatomy, merely to describe the structure of preserved dead things."—*Nature*, vol. 122, p. 444; *Science*, vol. 68, p. 287.

Diese Zellen treten 4, 5, 6, und 7seitig auf: eine Regel ist darüber nicht festzusetzen.—*Meyen, Phytotomie, 1830.*

Introduction.—This is a third report on the growth of cells in simple epithelium as indicated by their size and shape. The first[1] was an *a priori* consideration of geometrical necessities and probabilities, with an attempt at verification in the pigmented epithelium of the human retina. The second,[2] limited to measurements of cells in the single-layered, radially columnar, epidermal epithelium of a 100-mm. cucumber, justified the opinion that cell division is the antithesis of cell growth. After a cell has divided, those beside it usually grow, and the daughter cells also grow, but division itself is not growth. "Growth of the cell regularly alternates with cell division," for there is "a fundamental and ever present incompatibility between the principles of binary fission and least surfaces."[3] With that statement of Harper's we agree,

[1] The effect of cell division on the shape and size of hexagonal cells. Anat. Rec., 1926, vol. 33, p. 331–355.

[2] The correlation between cell division and the shapes and sizes of prismatic cells in the epidermis of Cucumis. Anat. Rec., 1928, vol. 38, p. 341–376.

[3] Harper, R. A. Binary fission and surface tension in the development of the colony of Volvox. Mem. Brooklyn Bot. Garden, 1918, vol. 1, p. 154 and p. 165.

and with D'Arcy Thompson when he writes,—"The phenomenon of division of the cell will be precisely what is required to keep approximately constant the ratio between surface and mass, and to restore the balance between surface-energy and the other energies of the system." Unfortunately, however, he allows division to be "a somewhat peculiar kind of growth; it is the growth which is limited to increase of surface unaccompanied by growth in volume or mass,"[4]—that is to say, the "kind of growth" which occurs when a sphere is merely flattened. Among others, Hammett confusedly writes of "growth by increase in cell size (assimilative growth) and growth by increase in cell number (proliferative growth)"[5]; but the increase in cell number by division is not growth unless the daughter cells enlarge, when it is "assimilative growth."

If the prismatic cells of the cucumber-epithelium in the 100-mm. specimen are arbitrarily represented as spheres, it is possible to summarize the previous observations in a simple diagram. In figure 1, let *A* represent the resting cell of average volume, containing a spherical nucleus of the observed nucleo-cytoplasmic ratio 1 : 17, which ratio was assumed to be maintained throughout, since the measurements were not to the contrary. The average volume of the dividing cells was found to be 50 per cent. greater than that of resting cells. Accordingly, when cell *A* has grown to the size of *B*, it divides into daughter cells which average one-half the volume of the parent, or three-fourths the volume of the resting cell (fig. 1, *C*). Thus division reduces the size of the cells, but it causes, per unit of volume, an increase in their surface areas. If the average volumes of the resting, dividing, and daughter cells (and of their nuclei) are as 1 : 1.5 : .75, then their surface areas are as $1 : (\sqrt[3]{1\frac{1}{2}})^2 : (\sqrt[3]{\frac{3}{4}})^2$, or approximately as 1 : 1.3 : .8. The daughter cell, per unit of volume, has 25 per cent. more surface than the dividing cell.

[4] Thompson, D'Arcy W. Growth and Form, 1917, p. 35.

[5] Hammett, F. S. Cell division and cell growth in size. Protoplasma, 1929, vol. 7, p. 535

Another result of division is to bring the centrally placed nucleus nearer the surface. The distance between the nucleus and cell wall shown in *A* is increased by growth in *B* from 1 to $\sqrt[3]{1\frac{1}{2}}$, or by 14 per cent.; and is decreased by division in *C* to $\sqrt[3]{\frac{3}{4}}$, being 9 per cent. nearer the surface than in *A*, and 20 per cent. nearer than in *B*. Even though the nucleus should migrate from its central position and come into contact with the cell wall at some point, the average distance from the entire wall would still be reduced. So there are three outstanding morphological results of division,—

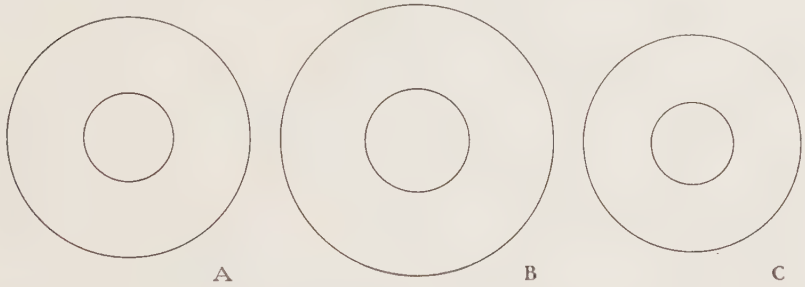

Fig. 1 Diagram showing the oscillation in volume between the average resting cell, *A*, dividing cell, *B*, and daughter cell, *C*, in the epidermal epithelium of the cucumber 100 mm. in length (based upon data tabulated in Anat. Rec., vol. 8, p. 369, fig. 10). Volumes of *A*, *B*, and *C* are as 4: 6: 3. Nucleo-cytoplasmic ratio assumed to be constant, as 1: 17, but the volume of the nucleus should presumably be somewhat less in *B*, this new ratio being transmitted to *C*. × 2500 diam.

reduction in the size of the cell and its nucleus, relative increase in their surface areas, and a lessening of the distance between the nuclear membrane and the cell wall—all of which counteract the effects of cell growth.

Certain distinctive properties of the dimensions of the cells, too small to be measured by the methods used, do not appear in the diagram, figure 1. That diagram makes division a complete corrective for cell growth, so that cells of a full-grown cucumber would be more numerous, but no larger, than those of the stage studied. It is clear, from the condition in successive stages, that the cytoplasm grows somewhat faster

than the nucleus, so that the nucleo-cytoplasmic ratio becomes altered in the dividing cell; and whenever nucleus and cytoplasm divide in halves, the new ratio will be inherited by the daughter cells.[6] These criticisms of the diagram are based on the following observations.

Dimensions of the average epidermal cells in cucumbers of 60, 100, and 220 mm.—In a 60-mm. cucumber the average cross-sectional area of the epithelial cells was estimated by measuring with a planimeter five patches of these cells, in tangential sections of the rind, drawn × 750 diameters. The 284 cells included in these patches were of various shapes, but, as shown in the previous study, the average area of all shapes is the same as the average area of the hexagons alone. Consequently their average area, 40 sq.μ, may be taken as the area of the typical hexagonal cells in cross section. Since the height of these cells in vertical sections of the same tissue averages 13 μ, the volume of the average epithelial cell in the 60-mm. cucumber becomes 520 cu.μ. The slight curvature of the top plate, and the pointed base which many, but not all, of these cells possess, have been disregarded, since, as previously shown, the additions which they make are trivial.

For the cucumber 100 mm. long, the dimensions of the typical epidermal cell have already been recorded (Anat. Rec., vol. 38, p. 354). It is a hexagonal prism 45 sq.μ in cross section, 23.5 μ tall, with a volume of 1055 cu.μ. In the 220-mm. cucumber, the cross-sectional area of 100 hexagonal cells ranged between 75 and 178 sq.μ, with an average of 112 sq.μ. The height is 65 μ; and the volume, 7280 cu.μ.

[6] In The Anatomical Record, vol. 38, p. 374, an attempt was made to explain why large pentagonal cells divide when still larger octagons are at rest, by assuming that the pentagons have relatively more cytoplasm than the octagons. The reverse is probably true. Large cells presumably have relatively more cytoplasm than smaller ones, except that the small cells recently formed by division inherit the reduced nucleo-cytoplasmic ratio of their large mother cells. This inheritance will account for the steady decline in nucleo-cytoplasmic ratio as the cucumber grows, and with this decline the rate of division falls. The inference is that cytoplasmic development retards division in those cells which need it most, so that the correlation with cell size is not absolute. In Minot's sense, the octagonal cell at rest when the smaller pentagonal cell divides is already somewhat 'senescent.'

From these measurements the diagram, figure 2, has been constructed, showing on the same scale the typical epithelial cell of the 60, 100, and 220-mm. cucumber. Although in the 60-mm. specimen some exceptional cells are still nearly isodiametric, the radial elongation has already set in. In these radially directed prisms, the top and basal surfaces are to become far removed from the central nucleus. There is no indication that the radial dimension, on which the great size of the cells in the mature cucumber so largely depends, has

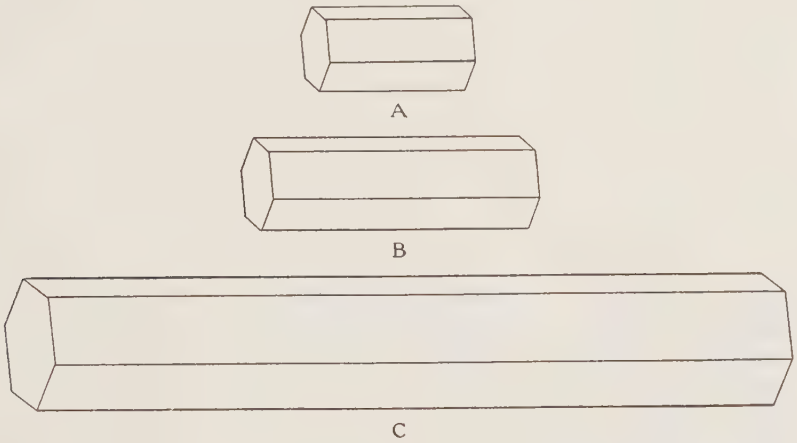

Fig. 2 Projections of prisms having the form and proportions of the average epidermal cell in cucumbers 60 mm. long, *A;* 100 mm. long, *B;* and 220 mm. long, *C*. The rounded top and facetted base of certain cells (*cf.* Anat. Rec., vol. 38, p. 356, fig. 5) have been omitted. × 1500 diam.

any effect on cell division. Division never reduces that dimension, which is approximately uniform for all the cells in a given area. Cells of the same height vary in girth, however, and division reduces their area in cross section, selecting the large ones.

Cross sections of the cells shown in figure 2 have been drawn on a larger scale in figure 3. The measurement of the nucleus is merely an approximation. Its diameter in the 60-mm. cucumber averages perhaps 4.5 μ, corresponding with a volume of 48 cu.μ and a nucleo-cytoplasmic ratio of 1 : 10.

In the 100-mm. stage we have reported the ratio as 1 : 17, making the nucleus 59 cu.μ in volume, with a diameter of 4.7 μ. At 220 mm. the diameters of ten typical nuclei averaged 6.13 μ; the nuclear volume is then 119 cu.μ, and the nucleo-cytoplasmic ratio 1 : 60. The cuticula in all cases has been included with the cytoplasm, together with everything (except the nucleus) which is within the cell wall.[7] The measure-

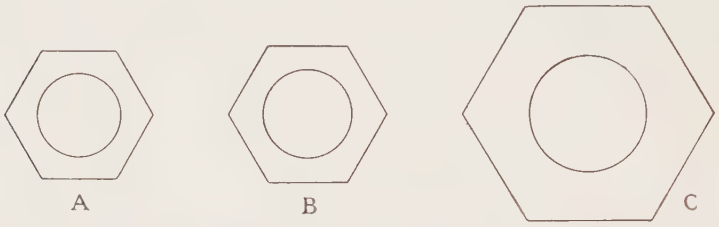

Fig. 3 Cross sections of the average epidermal cell in cucumbers 60 mm. long, *A;* 100 mm. long, *B;* and 220 mm. long, *C*. The nucleo-cytoplasmic ratio in *A* is 1 : 10. In *B*, 1 : 17. In *C*, 1 : 60. × 2500 diam.

ments indicate that the nucleus enlarges with the growth of the cell, but not at the rate of cytoplasmic increase. The dimensions correlated with cell division are those shown in figure 3. Cells which enlarge in that plane, in any diameter of the polygon, so that they are of greater area than their fellows, are the ones most likely to divide. It is a question of *relative* size. Cells of dimensions which make them large in the young cucumber, divide; whereas those of similar dimensions in the older cucumber are small and continue to grow.

[7] B. F. Lutman (Senescence and rejuvenescence in the cells of the potato plant. Vermont Agric. Exp. Sta., Bull. 252) states that "the amount of cytoplasm present in younger cells would seem to be greater than that in the older ones, although the bulk is very difficult to estimate. . . . It is obviously impossible to work out a nuclear-cytoplasmic ratio of different aged cells, such as Minot attempted in animal cells, when such a large part of the cell bulk is made up of vacuoles" (p. 18–19). He then tabulates the "proportion of nucleus to cell" as a substitute. But the ratio between nuclear volume and that of the rest of the cell is the nucleo-cytoplasmic ratio of young stages, and we prefer to apply it to old cells also. Professor Lutman's determinations of the nucleolar volume are without deductions for the "enormous vacuoles" which he finds within them. Ours of the cytoplasm also include "enormous vacuoles."

It is interesting that my former associate, Dr. Hammett, writing from the viewpoint that "cell division, like any other living process, is the visible expression of chemical reactions," warns the "unwary" not to "conclude cell size is a determining factor in rate of cell division." "Both large and small cells," he reports as common observation, "are to be seen dividing even in morphologically homologous regions." He has not computed the infrequency of divisions among the small ones. There may well be, as he believes, "an essential chemical grouping stimulative of cell multiplication." If so, the morphologist suggests that it is likely to be found in the many-sided cells of the epidermis of the cucumber, which accordingly are large in cross section, the probability of its occurrence increasing with the number of sides. In the 100-mm. cucumber we have estimated that division is 250 times as likely to occur in a 9-sided cell as in a 5-sided, the former being 50 per cent. larger than the latter. Other curious loci for the activity of "sulfhydryl, the essential stimulus to growth by increase in cell number" (Hammett: Protoplasma, 1929, vol. 7, p. 320) will be reported in Tradescantia.

Relation between epithelial growth and the growth of the entire cucumber.—Although there are considerable irregularities in the form of these cucumbers, such as curvatures and gourd-like enlargements at one end, as a rule the shape seems established at the 60-mm. stage, so that subsequent growth is an even expansion in all directions. It is irrelevant to this study how the form at 60 mm. has arisen. Avoiding the irregular shapes, two straight cucumbers of each size studied were selected, and the averaged measurements of these three pairs are as follows:

Length	60 mm.	100 mm.	220 mm.
Maximum diameter	11.5 mm.	18.5 mm.	42 mm.
Surface	1850 sq.mm.	4955 sq.mm.	26225 sq.mm.
Volume	5 cc.	20 cc.	240 cc.

These lengths are as 1 : 1.67 : 3.67 and the diameters vary in almost the same ratio,—1 : 1.61 : 3.65. The surfaces are approximately as the squares of these dimensions: the squares are as 1 : 2.7 : 13.4 and the surfaces as 1 : 2.7 : 14.2. The volumes are as the cubes of the linear dimensions. As compared with 1 : 4.4 : 49, the volumes are found to be 1 : 4 : 48.

Hence the expansion of the epithelial surface of the cucumber may be considered to occur uniformly in all directions.[8]

Neglecting the tubercles and other excrescences, and considering the surface as smooth throughout, it may be estimated from the data already presented that the numbers of cells covering the 60-mm., 100-mm., and 220-mm. cucumbers are respectively 46,250,000 cells, 110,000,000 cells, and 234,000,000 cells. The differences between these figures represent the numbers of mitoses between the successive stages,—63,750,000 between 60 and 100 mm., and 124,000,000 between 100 and 220 mm. Consequently every cell divides once and 38 per cent. divide twice in growing the first 40 mm.,; whereas every cell divides once and 13 per cent. divide twice in growing the last 120 mm. This is the expected great decline in mitotic activity. In the available sections of 210- and 220-mm. cucumbers, no mitoses were found.

Considered as a whole, the volume of the epithelial layer, as estimated by multiplying the surface area of the cucumber by the height of its epithelial cells, increases from 24 cu.mm. in the 60-mm. specimen to 117 cu.mm. in the 100-mm. stage, and to 1700 cu.mm. at 220 mm. In proportion to the volume of the whole cucumber, it rises from 1/200 to 1/170 and then to 1/140. The epithelium, therefore, is growing faster than the rest of the cucumber, and the excess is an addition to the cells in their radial dimension. If the volume of the epithelium in relation to that of the whole cucumber were the same at 220 mm. as at 60, the height of the cells would be 45 μ instead of 65 μ. They would still be columnar cells, relatively taller than at 60 mm.

The geometric features of this epithelium which seem correlated with the uniform spread of the epithelial surface in all directions, are the isodiametric shape of the cells in cross

[8] This accords with the findings of Sinnott and Durham for the different shapes of squashes. Each shape is distinguishable when the ovary is no more than 1 mm. wide, "often less than one-millionth of the volume of the fruit at maturity." "As development proceeds, growth in width keeps pace, proportionately, with growth in length, so that the ratio between these dimensions remains essentially constant" (Bot. Gaz., 1929, vol. 87, p. 418 and p. 416).

section (*i.e.*, in a plane tangential to the cucumber) and the random orientation of the polygonal cell-outlines in relation to the axes of the cucumber. Cell division is followed either by a circular expansion of the four cells chiefly involved, or, if this expansion is elliptical (*cf.* Anat. Rec., vol. 38, p. 360), the random arrangement of the long axes of the ellipses from repeated divisions neutralizes this effect, so that circular expansion results. The remainder of this paper is chiefly a measurement of growth of a very different type, in which the increase in length of the organ is far greater than the increase in breadth.

Growth in the internodes of Tradescantia virginiana.— The leaves of this plant arise singly along opposite sides of an upright stem, at slightly swollen nodes. Between the nodes the stretches of stem, or internodes, become successively shorter toward the top of the plant, and of smaller diameter, suggesting the decreasing segments of a collapsible spyglass. But the internodes taper somewhat, and as a rule, are not circular in cross section, since they tend to have a long axis toward the leaf-base and a short axis at right angles.

Fifty internodes were measured, taken from nine plants, and including the topmost, but never those nearest the ground. Their lengths (l) ranged from 28 to 210 mm.; and their circumferences (c), taken midway between the nodes, from 7.5 to 26 mm. The formula $l = 10c - 50$ expresses closely the average relation of length to circumference in the group measured, but variation is so great that for single instances it is quite unreliable. On the average, for one unit added to the circumference of the stem, ten units are added to its length. The epithelium covering these stems, though having many features in common with that of Cucumis when both are in their earliest stages, diverges rapidly. Whereas the epithelium of the cucumber becomes radially prismatic, that of Tradescantia becomes longitudinally prismatic, which is a type apparently not represented in the human body.

Epidermis and collenchyma of Tradescantia: general features.—The familiar aspect of this epithelium in surface view is seen in figure 4, based upon reconstruction from transverse sections. The underlying cells are shown at the top of the

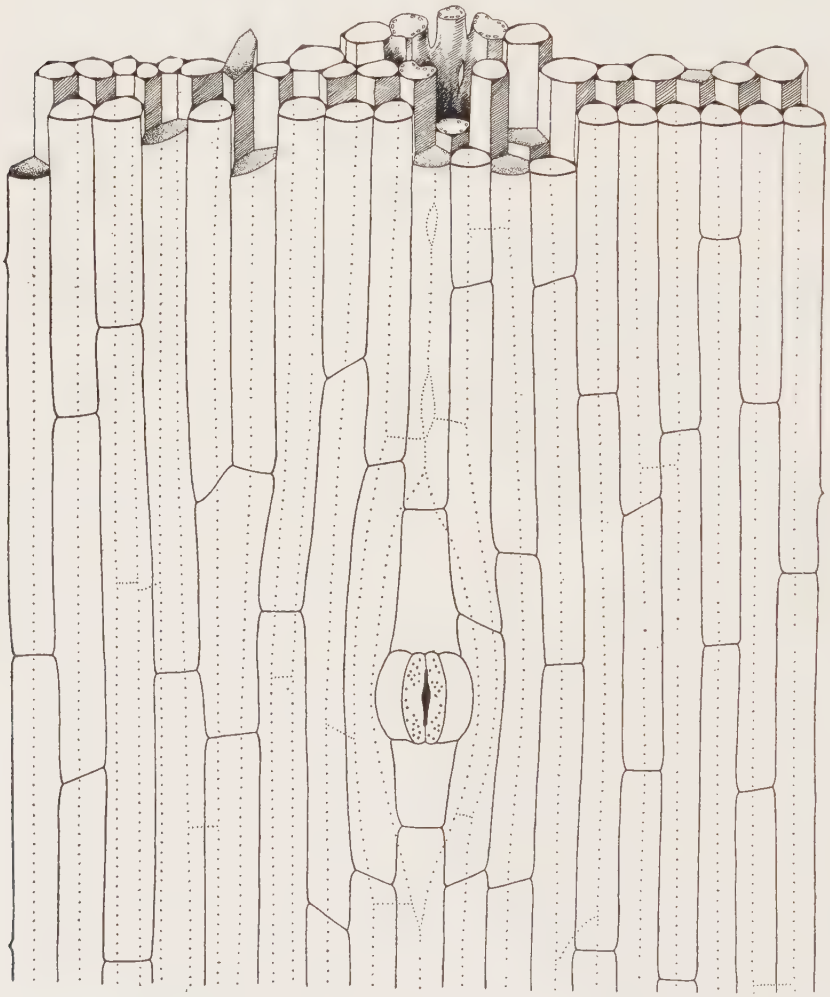

Fig. 4 The epidermal epithelium of *Tradescantia virginiana* in surface view, from a mature stem 11 mm. in circumference. The underlying collenchymal cells are shown at the top of the figure, and their downward extensions have been dotted. The dotted lines show the extent to which the epithelium is free from the collenchyma in the vicinity of the stoma. Reconstructed from transverse sections. × 200 diam.

drawing, and their downward extensions and transverse walls are indicated by dotted lines. The shape, size, and number of contacts of every epithelial cell with all its neighbors can thus be determined with precision. Similar pictures are more readily obtainable by merely stripping the outer layer from the stem and focusing at different levels. The underlying tissue, though closely packed and not stellate, bears an interesting analogy to mesenchyma. At the corners of the cells there are important but minute intercellular spaces, which become filled by localized thickenings of the cell wall. Two stages of this process are shown in the transverse sections *K* and *L* of figure 8 (p. 87), which include the epithelium above and the supporting tissue below. The venerable debate whether these thickenings develop within the cell membrane, or on its outer, intercellular-space side, parallels that concerning the intra- or extra-cellular origin of collagenous fibers. By fortunate coincidence this vegetable tissue, when the thickenings are limited to the corners of the cells, is termed collenchyma.

Link proposed *collenchyma* for sticky pollen cells (Elementa Philosophiae Botanicae, ed. altera, 1837, v. 2, p. 198): Schleiden, in 1839, said it was inappropriate, but whoever liked 'fastidious and superfluous terms' might apply it to the layer of thick-walled cells most highly developed beneath the epidermis of cacti (Anat. d. Cacteen, Mém. Acad. Imp., St. Pétersbourg, 1845, v. 4, p. 348). For Mohl, who figured this tissue in the beet, with thickened walls at the *corners* of the cells, it was the 'so-called collenchyma' (Anat. u. Physiol. d. veg. Zelle, 1851, p. 38); and Robert Brown (Manual, 1874) omits it, though listing ten or more terms ending in *enchyma,* with the comment:—"I have given the chief of these names in footnotes, so as to obviate as far as possible the mischief done by their injudicious adoption." But with Sach's Lehrbuch in 1868, figuring collenchyma in begonia petioles, the name became established—like prosenchyma, sclerenchyma, and many others.

In 1879, the Hertwigs introduced mesenchyma (German, Mesenchym) in their Cölomtheorie (p. 78). Balfour (Comp. Embr., 1881, v. 2, p. 296) translated it *mesenchyme,* as did Mark in his edition of Hertwig's Embryology in 1892. With such influential sanction that spelling became common, though Minot's Human Embryology of 1892 had it *mesenchyma.* Which of these two spellings, both in com-

mon use, should be adopted? The parent term is the παρέγχυμα of Erasistratus, Latinized as parenchyma (B.N.A.), and so spelled in English, with rarest exceptions, both by botanists and zoologists. The long list of derived botanical terms are all spelled with *a*. Consequently, if the spelling of every scientific term is not to be a law unto itself, mesenchyme should be replaced by mesenchyma in all well-edited textbooks and journals. It would be fortunate in other instances of divided usage, if the merits of the case were equally clear.

Although the collenchymal intercellular spaces are generally greatly reduced or obliterated, at quite regular intervals certain of them enlarge; and in vertical columns these widened spaces communicate with the exterior through stomata, each of which is bounded by two small epithelial cells containing chlorophyll (fig. 4). Cell division in the stomatal columns and those immediately adjacent is more frequent than elsewhere, and of a characteristic sort. Epithelium unaffected by proximity to the stomata will be considered first.

Form of average hexagonal cells in the epidermis of Tradescantia.—There are two typical ways in which a mosaic of regular hexagons can be oriented. The hexagons may be flat above and below, and interdigitate laterally; or they may interdigitate above and below, and be flat laterally. The same contrast occurs when the hexagons are vertically elongate. Link (Elementa, 1824) introduced his term *prosenchyma* for the cells which are pointed above and below, restricting parenchyma to those which are flat; but he failed to appreciate the geometrical nature of the distinction. He did not perceive that his parenchyma is prosenchymatous laterally, and that prosenchyma is parenchyma rotated 90°. The epidermal epithelium of Tradescantia has the 'parenchymal' arrangement. Its cells are in vertical columns with flat sides above and below, and since from a very early stage the two pairs of lateral sides also tend to become vertical, the hexagons become rectangular. The young cells in figure 6, *A* (p. 79), show this process in development. Schaffer attributes the rectangular form to growth in length—"Die Zellen

zeigen infolge des orientierten Längenwachstums vorwiegend rechteckige Formen"[9]—but it needs more than this. Hexagons lengthened indefinitely would be twice as wide in the middle as at the ends. The essential requisite to transform regular hexagons into rectangular hexagons is greater growth in breadth at the ends than in the middle.

Although the hexagonal cells become approximately rectangular, the sides seldom meet at right angles (fig. 4). More or less perfectly they retain angles of 120° by such curvature as Moldenhawer figured for Tradescantia,[10] and Berthold discussed for other plants in his Protoplasmamechanik (1886, p. 257)—"Wo drei . . . . Membranen zusammentreffen, sie sich so wölben und krümmen, dass die in dem gemeinsamen Schnittpunkte an sie gelegten Tangenten gleiche Winkel miteinander bilden."

The average cross section and the cell as a whole.—As seen in transverse sections of a mature stem 15 mm. in circumference, the cells, which are rectangular hexagons on surface view, are typically five-sided (fig. 5, *G*). If in the midst of similar cells they would doubtless have six sides, but two of these are replaced by the arched cuticula on the free surface. Averaging the measurements of 25 cells, the cuticula is the arc of a circle, subtended by an angle, at the center, of 76°. The lateral walls do not deviate perceptibly from the vertical, and the basal surface is typically 2-sided, with sides meeting at an average angle of 136°. The average thickness of these cells is 30 μ, breadth 28 μ, and area 720 sq.μ. Since the cells are 340 μ tall, as seen in longitudinal sections, and of essentially uniform cross section throughout, the volume of the cell is 245,000 cu.μ. An entire cell is projected in figure 5, *D*, with cells of the cucumber, *similarly oriented*, and drawn on the same scale in figure 5, *A*.

An average cell from near the node of an undeveloped stem 7.5 mm. in circumference is seen projected in figure 5, *B*, and

[9] Schaffer, J. Das Epithelgewebe. Hdb. d. mikr. Anat. d. Menschen, herausgegeben von W. von Möllendorff, 1927, Bd. 2, Teil 1, p. 1.

[10] Moldenhawer, J. J. P. Beyträge zur Anatomie der Pflanzen. Kiel, 1812, p. 98 u. Tab. V, fig. 4.

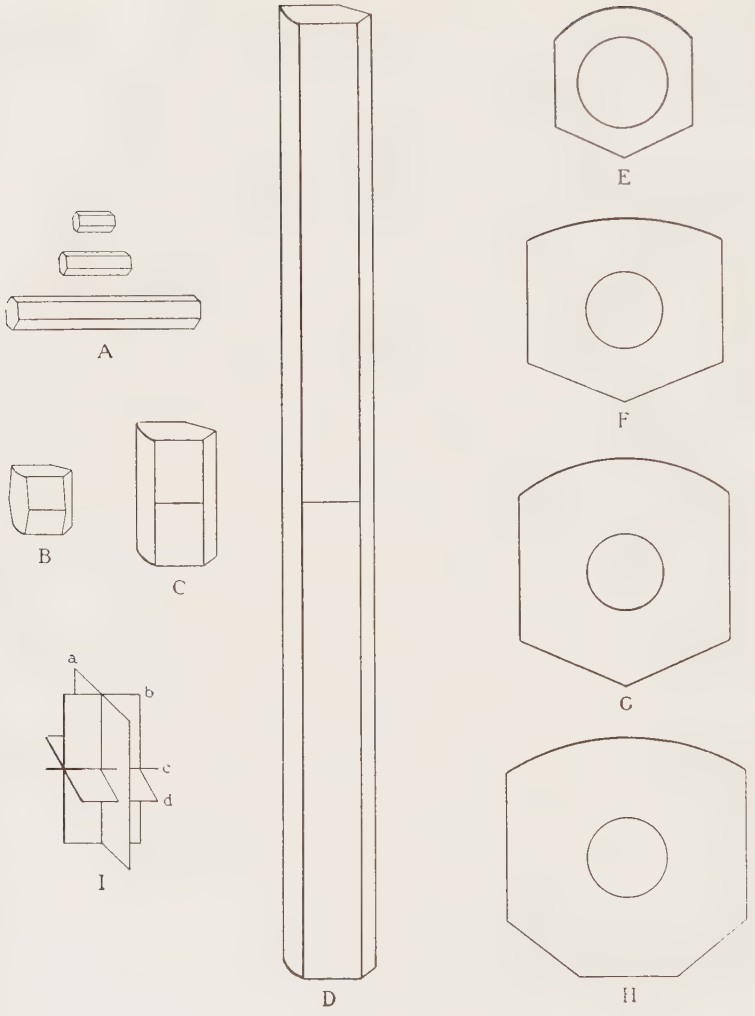

Fig. 5 *A*, epidermal cells of the cucumber, being figure 2 reduced to × 375 diam. *B–D*, projections of typical epidermal cells of *Tradescantia virginiana*, × 375 diam., oriented as in *A*, the vertical line being lengthwise of cucumber and stem respectively. *B–C*, from a young internode 7.5 mm. in circumference (*B*, toward the node); *D*, from a mature internode 15 mm. in circumference. Length of *B*, 21 μ; of *C*, 45 μ; of *D*, 340 μ. E–H, cross sections, × 1000 diam. *E*, section of *B*, width 18 μ, area 250 sq.μ. *F*, section of *C*, width 26 μ, area 540 sq.μ. *G*, section of *D*, width 28 μ, area 720 sq.μ. *H*, average section of cells with 3-sided bases associated with *G*, width 32 μ, area 900 sq.μ. Nuclear dimensions in text. *I*, the planes of cell division in a hexagonal cell oriented as in *A*: *a*, tangential; *b*, radial; *c* and *d*, obliquely transverse.

in section at *E:* its volume is 5200 cu.μ. A cell intermediate between *B* and *D* is shown in *C* and *F*. No significance is attached to the fact that this cell—the average of ten measured—is somewhat broader than thick, with a less arched cuticula. Considerable variation is to be expected.

Nucleo-cytoplasmic ratio.—The nuclei in the young epidermal cells of Tradescantia are very large (fig. 6, *A*). Fifteen of them measured in cross section ranged in area between 83 and 135 sq.μ, with an average of 110 sq.μ. This is the section of a sphere of 870 cu.μ, and the nucleo-cytoplasmic ratio of these young cells, hexagonal on surface view, is then 1:5. Fifteen cells heptagonal on surface view, and consequently larger, had nuclei ranging from 80 to 142 sq.μ in section, with an average of 110 sq.μ, precisely as in the case of the hexagons. This, however, means that their nucleo-cytoplasmic ratio has been reduced to 1:6. Fifteen cells pentagonal on surface view had nuclei from 57–107 sq.μ, with an average of 79 sq.μ and volume 524 cu.μ, the ratio being as 1:6. This is consistent with the inheritance of a diminished ratio by the daughter cells, but the great chance for error and the limited number of measurements make this conclusion tentative.

In the ten cells of larger size, presented in figure 5, *C* and *F*, all hexagonal on surface view, the areas of the nuclei are 71–100 sq.μ, average 84 sq.μ, volume 579 cu.μ, and ratio 1:41.

Twenty-five large cells, hexagonal on surface view and with 2-sided bases, had nuclei measuring 53–81 sq.μ, average 69 sq.μ, and volume 430 cu.μ. The average volume of these cells, somewhat less than that drawn in figure 5, *D* and *G*, was 195,000 cu.μ, making the nucleo-cytoplasmic ratio 1:450. The cytoplasmic volume here includes the cuticula, vacuoles, and all products of 'pseudo-growth,' being merely a measure of the size of the cell, with the nucleus deducted.

Besides cells with 2-sided bases, there is a smaller number of cells with 3-sided bases (fig. 5, *H*), the numerical occurrence and significance of which are discussed in a following section. These cells are of the same length as those with 2-sided bases (50 hexagonal cells with 3-sided bases average

337 μ in length: 100 hexagonal cells with 2-sided bases, 340 μ): but they are 25 per cent. larger in cross section (average of 75 measurements). They are 15 per cent. wider than cells with 2-sided bases. The arch of the cuticula is lower, on the average, being subtended by an angle of 64° instead of 76°. The 'vertical' sides, which often diverge from the cuticula to the base, articulate with corresponding sides of cells with 2-sided bases, yet there is apparently a tendency for such sides to be slightly longer in the cells with 3-sided bases, the difference being less than 1 μ. Of the three basal sides, the middle averages 16 μ, and the lateral 15 μ each, as compared with 19 μ for the edges of the 2-sided base.

Two series of measurements, in different stems, indicate that the nuclei of cells with 3-sided bases are slightly larger than those with 2-sided bases. In the series yielding figure 5, *G* and *H*, the nuclear area in *H* (average of 25 measurements) is 9 per cent. larger than in *G*, making the nuclear volume, in the cell with 3-sided base, 610 cu.μ, and the nucleo-cytoplasmic ratio 1:500. In the other series the difference in nuclear area was 2 per cent., involving a still greater reduction in ratio.

Growth in the cucumber, in the stages studied, is accompanied by a fall in nucleo-cytoplasmic ratio from 1:10 to 1:60. In the more or less comparable stages of Tradescantia the decline is from 1:5 to 1:450. Contributing to this great reduction there is an actual diminution in the size of the nucleus. In the cucumber the nucleus steadily enlarges as the cell grows, but not at the rate of cytoplasmic increase. In Tradescantia, prepared in the same way, the nucleus in older stems is certainly smaller than in the young stages. This need not be due to condensation or loss of nuclear material, since division reduces the size of the nucleus, and slower growth of the nucleus after division, as compared with the cytoplasm, would yield the same result. Possibly related to the difference in the behavior of the nucleus is the fact that in Cucumis the epithelium grows faster than the underlying tissue, whereas in Tradescantia the reverse is the case.

Growth of the epidermis in Tradescantia as compared with the growth of the stem as a whole.—The cross section of a young stem, 7.5 mm. in circumference, has an area of 4.3 sq.mm., of which its epithelium, measuring 156,000 sq.μ, constitutes 3.6 per cent. A stem 9.9 mm. in circumference, 7.7 sq.mm. in section, has an epithelium of area 245,000 sq.μ, being 3.2 per cent. of the entire cross section. An autumnal internode, 14.8 mm. in circumference, is 16.7 sq.mm. in section, of which the epithelium forms 361,000 sq.μ, or 2.1 per cent. Thus, contrary to the conditions in the cucumber, in which the epithelial growth exceeds that of the tissues beneath, the epithelium here falls behind and becomes a relatively thinner covering as the stem enlarges. Its actual average thickness increases slightly, being 20.8 μ, 22.3 μ, and 24.3 μ in the three successive stages, whereas that of the cucumber increased from 13 to 65 μ. This dimension in neither case is affected by cell division.

In the cucumber, cell divisions limit equally the enlargement of the cells both transversely and longitudinally in relation to the long axis of the cucumber, and keep them isodiametric. In Tradescantia, vertical divisions restrict the transverse enlargement of the cells even more closely than in the cucumber. There are 290 cells around the 7.5-mm. stem, 350 around the 9.9-mm. stem, and 530 around that of 14.8 mm., indicating that after a slight increase in the width of the cell in early stages, vertical divisions maintain a constant width. But in Tradescantia the length of the cell is allowed to increase with the elongation of the stem, unrestricted, except in early stages, by transverse cell division. Some of the long cells in the 7.5-mm. stem measured 45 μ; in the 9.9-mm. stem there was an area of cells averaging 128 μ in length; and cells 340 μ long in the 14.8-mm. stem have already been noted. It would indeed be instructive to know why these cells fail to divide transversely.

It must not be inferred that the relatively slow growth of the epithelium in Tradescantia is due to infrequent divisions and the consequent large size of the cells. In the leaf-stalks

of the sugar maple the epithelium decreases from 4.5 per cent. of the cross-sectional area of a petiole 30 mm. long, to 1 per cent. of the cross-sectional area in the 95-mm. petiole. Here it falls behind the other tissues decidedly more than in Tradescantia, yet in this case frequent division keeps the cells small. Sinnott, whose recent study has directed attention to growth in the maple petioles, finds no increase in the size of the epidermal cells as the stems grow larger, but merely an increase in their number.[11]

There may be, then, a slow-growing epithelium of large cells (up to 245,000 cu.μ), due to infrequent division, as in Tradescantia: a slow-growing epithelium of small cells (2500 cu.μ), due to frequent division, as in Acer: a rapidly growing epithelium of rather small cells (attaining 7300 cu.μ) due to quite frequent division, as in Cucumis: and doubtless examples may be found of rapid growth and large cells, from infrequent division, which would complete the possible combinations.

The planes of cell division.—Of the infinite number of planes by which a hexagonal prism, oriented like the epithelial cells of the cucumber (fig. 5, *A*) can be divided in halves, surface tension, or the law of minimal surfaces, reduces the number to four, namely the planes, *a, b, c,* and *d* of figure 5, *I*. If the cell is considered as a member of an hexagonal mosaic, there is, in all cases, a maximum avoidance of 4-rayed intersections. In the cellular mosaic, every hexagon tends toward regularity, all deviation from which is an approach toward a 4-rayed intersection.[12] A new wall introduced by division should therefore divide opposite walls in

[11] This is his general conclusion. (Sinnot, E. W., The morphogenetic relationships between cell and organ in the petiole of Acer. Bull. Torrey Bot. Club, 1930, vol. 57, p. 1–20.) But he found a slight increase in length of the epidermal cells in the longer petioles, which is "only thrice its probable error and thus of very doubtful significance" (p. 11). Testing this by careful measurement of the epidermal cells in a single pair of petioles, 30 and 95 mm. long respectively, I find the volume of the epithelial cells 11 per cent. greater in the latter—a trivial increase as compared with that in Tradescantia.

[12] *Cf.:* A principle for determining the shapes of irregular cells. Anat. Rec., 1929, vol. 42, p. 27.

halves, this also being the maximum avoidance of the 4-rayed vertex. Although this is so far from being attained that 4-rayed vertices are not infrequent among certain cells, the tendency is always present, and this is what makes the average form of cells geometrically presentable.

In addition to the factor of minimal surfaces, there is a second factor determining the plane of division, more interesting because less understood. This unknown factor eliminates any general division in the tangential plane, both in the epithelium of the cucumber and in that of Tradescantia. In the former, the cell wall in this plane would be of much less area than any actually formed. In Tradescantia, it would be a very extensive wall. The area of the septum has therefore nothing to do with its non-occurrence; nor has the direction of predominant growth of the cell, which in one case is parallel with it, and in the other at right angles. If general division occurred in the tangential plane, the epithelium would become stratified.[13] Sporadically it does occur, producing multicellular plant-hairs in the cucumber and occasional unicellular forms in Tradescantia.

The radial plane (*b* of fig. 5, *I*) can be utilized by cells with the parenchymal orientation, as contrasted with the prosenchymal, provided that they have a flat basal surface. Cell *H* of figure 5 can divide strictly in that plane, whereas cell *G*, with its wedge-shaped base can not,—it would produce a 4-rayed intersection. Accordingly in *G*, if the plane is strictly radial, *i.e.*, parallel with the vertical sides, it must be so placed as to divide the cell unequally. If the division is to be equal, the plane must be obliquely radial; and the cells seem to compromise between these alternatives. Unequal divisions, long recognized in the cleavage stages,[14] are so com-

[13] The fact that in animals "a diet deficient in fat soluble A vitamin" may cause a simple epithelium to become stratified (Wilson, J. A., and Dubois, R. O., Amer. Jour. of Diseases of Children, 1923, vol. 26, p. 431–446) does not go far in clarifying the situation.

[14] "It is very noticeable that the cleavage furrows do not necessarily cut the mass which is segmenting through its shortest axis, any more than through its middle. It is very common to see a strip or sheet cut off from the side of a larger mass. . . ." Harper, R. A. Cell and nuclear division in Fuligo varians. Bot. Gaz., 1900, vol. 30, p. 217–251.

mon in these tissues as to be perhaps the rule. Oblique septa are also common. But cells with 3-sided bases particularly invite division in this plane, being large in area, and capable of exact bisection in a strictly radial plane.

Divisions in planes *c* and *d* of figure 5, *I*, occur typically in the cucumber, but merge in a simple transverse division, at right angles with plane *b*, in the rectangular hexagons of Tradescantia, avoiding, however, 4-rayed intersections. The transverse walls in Tradescantia are usually somewhat oblique, and occasionally are very sharply inclined.

The upper angle at which 100 transverse walls in Tradescantia, taken at random, met the vertical wall on their right, disregarding the notches previously described, varied from 35 to 140°, with an average of 89.70. Only six were less than 60°, and seven more than 120°; twenty might be considered crossing at right angles. Although the obliquity is ordinarily such as would come from avoiding 4-rayed intersections, in many cases the reverse is true. Instances of both sorts may be found in figure 4.

In summary, the isodiametric hexagons of Cucumis divide, perhaps to an equal extent, in planes *b*, *c*, and *d* of figure 5, *I*. The rectangular hexagons of Tradescantia tend to substitute a transverse plane for *c* and *d*. They are so organized that they divide transversely only when large by reason of their length; and vertically only when large by reason of breadth,—factors which are not separable in the isodiametric, promiscuously oriented cells of Cucumis. Increased size dependent on the radial dimension is apparently not a factor in cell division in either type of epithelium.

Transverse division in the epidermis of Tradescantia.—As in Cucumis, the invariable result of a single cell-division is to add six sides to the hexagonal mosaic. The two daughter cells together have four more sides than the parent, and two adjacent cells each receive an added side,—a geometrical necessity when there are no 4-rayed vertices. The results of transverse division are shown in the diagram, figure 6, *B*, beside which there is a group of actual cells for comparison. A rectangular hexagon, after a certain increase in length, has

divided into the hexagonal cell, *a*, and the quadrilateral, *b*, thus adding four sides to the mosaic. At the same time two adjacent hexagons, *c* and *d*, have been made heptagons.

In the cucumber the area of the cells has been found to vary as the number of sides minus two. Assuming that this holds true for the rectangular hexagons of Tradescantia, and that transverse division is independent of the width of the cell, which it does not affect in any way, then, when trans-

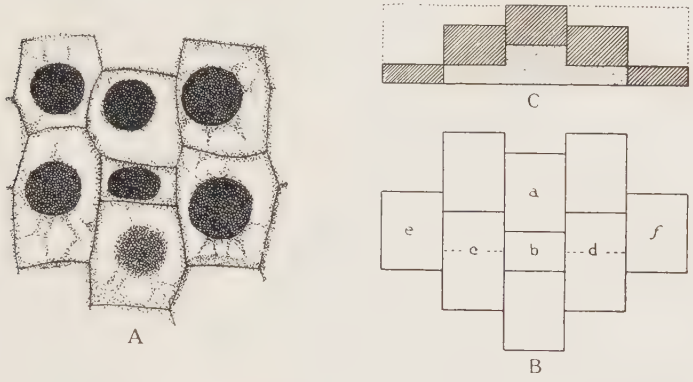

Fig. 6 *A*, epidermal cells of Tradescantia, toward the node of the young stem 7.5 mm. in circumference, for comparison with *B*. × 600 diam. *B*, diagram of the spatial results of the transverse division of a hexagonal cell into daughter cells *a* and *b*. *C*, diagram in which the stippled area represents the upward growth normally following the transverse division of a single cell in the central column. The cross-hatched area marks the upward growth following the transverse divisions of cells *c* and *d* in *B*; the dotted line marks the upward growth from a single transverse division in every vertical column.

verse division is being considered, the two sides to be deducted are those at the top and bottom. In other words, the width may be considered constant. The area of the cells then varies as their length, and the length as the number of lateral contacts. Hexagonal cells have four lateral contacts, and their length may be arbitrarily fixed at 4 units; the length of the quadrilateral cell is then 2 units; and of the heptagonal cell, 5 units. Eventually the result of the single division of the hexagon into *a* and *b* will be an upward growth of 2 units

in its own column, and of 1 unit in the column on either side, as indicated by the stippled area in figure 6, *C*. But the heptagons *c* and *d*, being large cells, may be expected to divide, in the plane of the dotted lines. The upward growth which follows these two divisions is shown by the cross-hatched area in *C*. Cells *e* and *f*, as heptagons, then divide, making other heptagons laterally; and when this process has encircled the stem, and one cell in every column has divided transversely, there would be a uniform upward growth of 4 units, to the level of the dotted line at the top of *C*. Every transverse division, whatever the number of sides of the parent cell, should be followed by an upward growth of 2 units in its own column, and of 1 unit in each of the adjacent lateral columns, the total effect being the addition of the area of one hexagonal cell to the entire mosaic.

The data on which the foregoing description of transverse division rests are as follows. That the width of the cells is unaffected by their length, at any given stage, can be seen in the freshly stripped epithelium. When a long cell is followed by a short one, there is no appreciable change in the breadth of the column (*cf.* fig. 4). In the young stem, the average widths of 25 pentagons, hexagons, and heptagons were found to be 18 μ, 19 μ, and 19 μ respectively. From two old stems the average widths, at the level of the nucleus, of 20 pentagons, hexagons, and heptagons, all with 2-sided bases, were respectively 27.5 μ, 27 μ, and 27 μ. Cells with 3-sided bases average wider than those with 2-sided, but they are not longer, as shown in the second section of table 1.

That the length of the cells varies as their number of lateral contacts is shown in table 1, first for young cells, and then for those in mature stems. The cells in growing 16 times as long maintain the same relative lengths. In the table the lengths are expressed in microns, and also in 'units' for more convenient comparison. The unit for any stage is obtained by dividing the average length of its resting hexagons by four, so that the average length of the resting hexagon has arbitrarily been made precisely 4 units. Then,

if the length of the cells varies as their number of lateral contacts, the length of the pentagon should be 3 units; of the heptagon, 5 units; etc. The small size of the octagons in the young stem—5.1 units instead of 6.0—seems due to insufficient data, though in the cucumber the many-sided cells fail to attain the full size required by the formula. On the whole, the formula is sufficiently verified.

TABLE 1

Length of cells in the epidermis of Tradescantia virginiana (this dimension being lengthwise of the stem)

I. Upper internode in spring: circumference 7.5 mm.

SHAPE OF CELLS ON SURFACE VIEW	RESTING CELLS			DIVIDING CELLS		
	Number measured	Range	Average	Number measured	Range	Average
Quadrilaterals	8	8.0–15.3 μ	10.8 μ			
(*Length÷5.2 in italics*)		*1.5– 3.0*	*2.1*			
Pentagons	25	12.0–21.3 μ	15.6 μ			
		2.3– 4.1	*3.0*			
Hexagons	100	10.7–31.3 μ	20.8 μ	9	24.0–32.7 μ	27.2 μ
		2.1– 6.0	*4.0*		*4.6– 6.3*	*5.2*
Heptagons	25	14.0–35.3 μ	25.1 μ	13	26.7–36.5 μ	31.4 μ
		2.7– 6.8	*4.8*		*5.1– 7.0*	*6.0*
Octagons	4	22.0–29.3 μ	26.3 μ	1	40 μ	
		4.2– 5.6	*5.1*		*7.7*	

II. Mature internode in autumn: circumference 14.8 mm.

SHAPE OF CELLS ON SURFACE VIEW	TWO-SIDED BASES (fig. 5, *G*)			THREE-SIDED BASES (fig. 5, *H*)		
	Number measured	Range	Average	Number measured	Range	Average
Quadrilaterals	5	130–285 μ	213 μ			
(*Length÷85 in italics*)		*1.5–3.4*	*2.5*			
Pentagons	25	185–330μ	268 μ	22	195–315 μ	256 μ
		2.2–3.9	*3.2*		*2.3–3.7*	*3.0*
Hexagons	100	240–490 μ	340 μ	50	255–430 μ	337 μ
		2.8–5.9	*4.0*		*3.0–5.1*	*4.0*
Heptagons	25	285–520 μ	426 μ	5	428–495 μ	467 μ
		3.4–6.1	*5.0*		*5.0–5.8*	*5.5*
Octagons	9	435–660 μ	527 μ			
		5.1–7.8	*6.2*			

That dividing cells are larger, on the average, than those at rest is shown also in the first section of table 1. Twenty-five measurable cells in mitosis were available. None of these was a quadrilateral or pentagon; 13 were heptagons, and there were two nonagons which are not included in the table. The average number of sides of these 25 dividing cells is 6.8, as compared with 6.99 for a thousand dividing cells in the cucumber. Evidently the average dividing cell in these epithelia is heptagonal, though the average resting cell is hexagonal. The average area of the 25 dividing cells in Tradescantia was found to be 5.9 units, which accords with the observation in the cucumber, that the average dividing cell is 50 per cent. larger than the average resting cell, namely the hexagonal cell of 4 units.

Longitudinal division in the epidermis of Tradescantia.— Just as the width of the cells has no influence on transverse division, the length is of no consequence in longitudinal division, and must be disregarded. It has been assumed, in making the diagram figure 7, *A*, that all six sides of the rectangular hexagons are of the same length, though the cells of Tradescantia do not pause at these proportions which they pass through in becoming elongate. Cell *a* in figure 7, *A*, widens to a certain extent and then divides longitudinally into a pair of pentagons (*b* and *c* of fig. 7, *B*), at the same time making heptagons of the hexagons above and below (*d* and *e*). If, as in the case of transverse division, the dividing cell is 50 per cent. larger than the resting cell, and the width of the resting hexagon is considered 1 unit, then the width of the dividing cell is 1.5 units. The width of each daughter cell is .75; and its area, in comparison with that of the hexagonal cell which is arbitrarily fixed at 4 units, is 3 units. The area of each of the heptagons *c* and *d*, 1.5 wide at one end, and 1.0 at the other, is 5 units. These areas are the same as those of the pentagons and heptagons produced by transverse division.

The narrow ends of the heptagons then widen to a breadth of 1.5 and divide lengthwise, *e* of figure 7, *B*, into *h* and *i* of

figure 7, *C*, changing the hexagon below into a heptagon, *j*. A 4-rayed intersection between *b*, *c*, *h*, and *i* may be avoided by a slipping of the walls in the manner shown in *D*, *c* and *h* becoming hexagons. In the epidermis of the maple, actual 4-rayed intersections are sometimes seen in this situation, together with all degrees of slight departure. Hexagons *c* and *h* then widen till they acquire the normal dimensions for hexagons and have an area of 4 units each. These changes

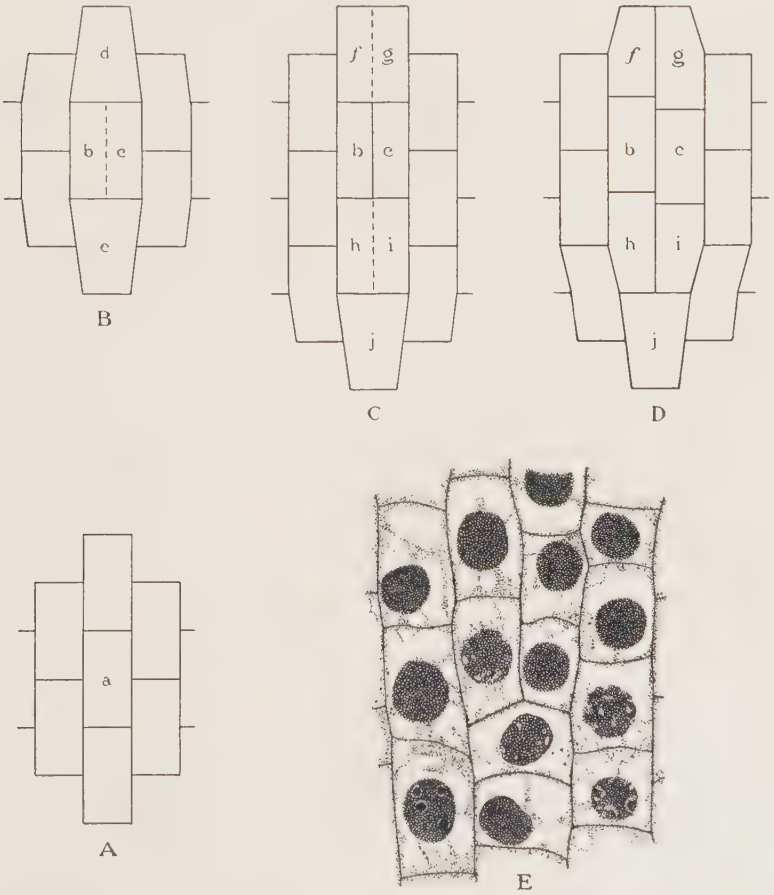

Fig. 7 *A–D*, diagrams of vertical division and subsequent growth in a mosaic of rectangular hexagons. *E*, epidermal cells of a young stem of Tradescantia for comparison with *D*. × 600 diam.

affect cells *f* and *g* in the same way as *h* and *i*, and bring it about that the cells are all hexagonal except the two pentagons *f* and *i*, and two heptagons, *j* and a corresponding cell, not drawn, above *f* and *g*. Thus a column of hexagonal cells is being split into two columns of hexagonal cells of normal size. Entire columns tend to split in Tradescantia, leaving adjoining columns at rest; but in the maple, the splitting shifts from one column to another, not going far in any, and contributing to the disarranged appearance of its mosaic.

The diagrams illustrating longitudinal division (fig. 7, *A–D*) are decidedly schematic. In the actual epithelium, however, groups of cells may be found, as in figure 7, *E*, in which cells *b*, *c*, *h*, *i*, and *j* of *D* can readily be identified. By a rather laborious method the average width of the cells in such groups was determined. The average width of 30 cells in the columns bordering laterally on the dividing column was found to be 18 μ. The average width of 10 cells just below those dividing (corresponding with *j* in fig. 7, *C* and *D*) was 22 μ; and the average width of 20 presumably recently formed cells in the position of *h* and *i* was 14 μ. According to the diagram, if the width of the resting cell is fixed at 4 units, that of the dividing cell (*j*) is 5, and that of the daughter cells is at first 3, later becoming 4. The expected proportion between the breadth of resting cell, dividing cell, and daughter cell is therefore as 4 : 5 : 3+ or as 36 : 45 : 27+. The proportions as measured in actual cells are as 18 : 22 : 14, or as 36 : 44 : 28. The diagram may be accepted, therefore, as at least an approximation to what is taking place.

Significant cell patterns.—Figure 8, *A*, shows diagrammatically an epithelium of cells with 2-sided bases resting on a hexagonal supporting tissue. Let cell *a* divide radially avoiding the 4-rayed vertex, along the dotted line, and it produces cells *b* and *c* of figure 8, *B*. A cell with a 1-sided base has been introduced into the epithelium: the subepithelial cell *d* has received an added side and grows accordingly. Unless the underlying cells are to become larger than those in the epithelium (which is often the case) cell *d* would be

expected to divide in the plane of the dotted line, whereupon cell *b* acquires a 2-sided base, and the original uniformity of the epithelial cells is restored. Meanwhile the deeper cell *g* has received an added side, and, having enlarged, may divide (as indicated in *C*), making the subepithelial layer again uniformly hexagonal. Delayed division in the interior, as compared with that toward the surface leads to the prevalent condition of large pith cells, small epidermal cells, and intermediate subepithelial cells.[15]

A further detail shown in the diagram is the result of the division of epithelial cell *m* (fig. 8, *B*) in a radial plane directed toward cell *d*: *m* thus produces *n* and *o* of figure 8, *C*, and *d* becomes *d'*. Groups of this sort are not infrequent in certain epithelia, and the way in which they may be resolved into the primary pattern of *A* is self-evident.

In contrast with the patterns *A–C*, in which division is initiated in the epithelial layer, there are the patterns *D–F*, in which division begins in the subepithelial layer. In figure 8, *D*, cell *d* divides into *e* and *f* of diagram *E*. Then epithelial cell *a* acquires a 3-sided base. It is a wide cell, and may be expected to divide vertically (into *b* and *c* of *F*), thus restoring the original uniformity of the epithelial layer, and duplicating the pattern already considered in figure 8, *C*.

The difference illustrated in the two series *A–C* and *D–E* is seen in the actual tissues. The epithelium of the full-grown cucumber shows, among 500 cells, 62 per cent. with 2-sided bases, 36 per cent. with 1-sided, and 2 per cent. with 3-sided, thus presenting the patterns *A–C*, and indicating that division is initiated in the epithelium. A count of 500 epithelial cells in Tradescantia shows 75 per cent. with 2-sided bases, 1 per cent. 1-sided, and 24 per cent. 3-sided. Division is therefore initiated in the collenchyma. Division may arise in both layers, as suggested by the epithelium surrounding the 30-mm. maple petiole. Its 270 epithelial cells included 44

[15] In maple petioles, as Sinnott expresses it, "there is obviously an enormous difference in dimensions between the cells of the epidermis and those of the pith, the latter averaging nearly one hundred times as great in volume as the former, with the two outer cortical layers intermediate in size" (*l.c.*, p. 7).

with 1-sided bases, and 19 with 3-sided: but division continues predominantly in the epithelium, so that in the long petiole, surrounded by 464 epidermal cells, there are 139 with 1-sided bases and only 6 with 3-sided. Since every 1-sided base in the epithelium means an additional cell as compared with the layer beneath, and every 3-sided base means one less cell than in the layer beneath, it is evident that the 464 epithelial cells rested on 331 subepithelial cells. In the slow-growing epithelium of the maple, longitudinal division is therefore initiated in the epithelium, just as in the rapidly growing epithelium of the cucumber: in Tradescantia it begins in the collenchyma, as is conclusively shown by the shapes of the cells.

Tangential divisions, absent from the epithelium of Tradescantia except in plant-hair formation, occur abundantly in the collenchyma, and give rise to very beautiful patterns when they chance to be limited to every other cell. In *G* of figure 8, let alternate hexagonal collenchymal cells divide in the tangential plane as indicated by the dotted lines. The result is shown in *H*,—superimposed pairs of pentagons alternating with octagons, precisely as in the actual section, figure 8, *K*. If now the octagons divide unequally as indicated in *H*, the singular pattern in *I* results,—pentagon over heptagon alternating with heptagon over pentagon. Heptagons, being larger, divide rather than pentagons; and those near the surface somewhat more readily than those deeper in. Let the outer heptagons divide tangentially as indicated in *I*. Each dividing heptagon thus produces a pentagon toward the epithelium, and a hexagon beneath the pentagon. At the same time the alternating pentagons are made heptagons (fig. 8, *J*). Should they divide in turn in the same way, the pattern is seen to be self-perpetuating, and serving for the radial expansion of the stem. It is only in small areas and by chance that precisely this disposition of the cells is found, but the group shown in *L* is exactly interpreted by the diagram *J*.

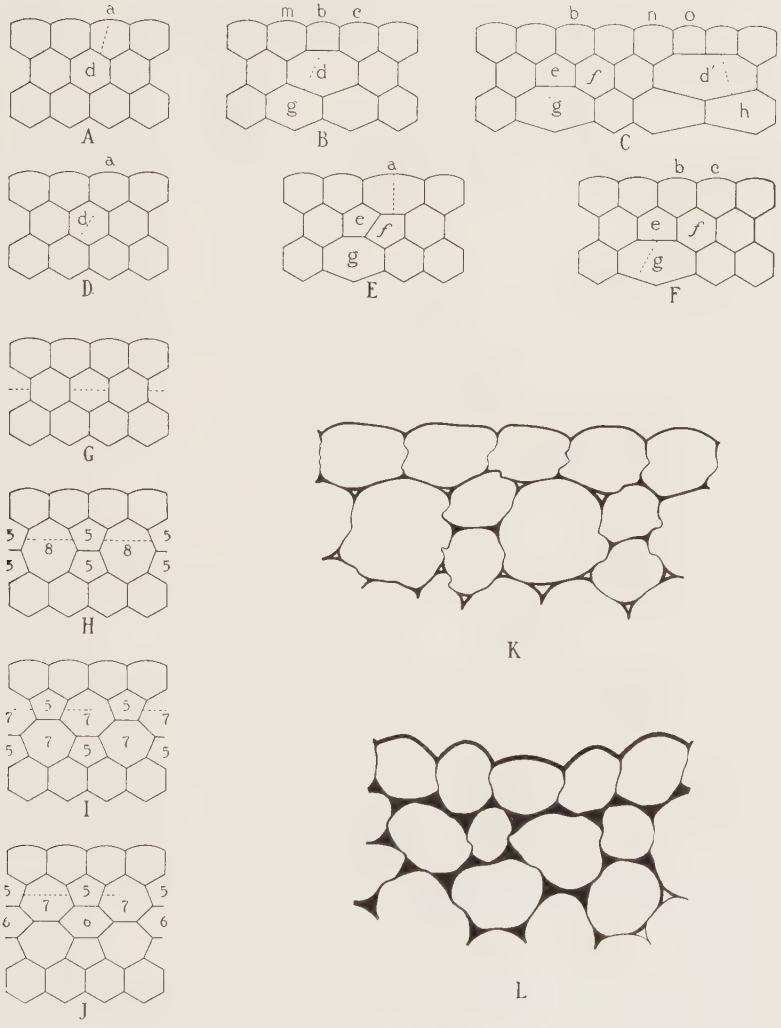

Fig. 8 *A–C*, cell patterns produced by vertical division initiated in the epithelium: *D–F*, those due to vertical division initiated in the subepithelial layer. *G–J*, patterns due to the tangential division of alternate subepithelial cells. *K*, group of cells (interpreted by *G* and *H*) from an immature Tradescantia stem. × 300 diam. *L*, group (interpreted by *J*) from a mature stem. × 300 diam.

Growth and division in the stomatal columns.—"It is clear enough that the last stage in the development of a stoma, is, from the physical point of view, not yet properly understood."[16] Perhaps also the younger stages, in which rules for growth and division applicable to the rest of the epithelium are entirely set aside! As seen in surface views, the first sign of stomata is the production of low quadrilateral cells, comparable with the one shown in figure 6, *A*. In the general epithelium such quadrilaterals grow, and by receiving added sides, become ordinary hexagonal cells. Of 100 quadrilateral cells which had become stomata, only 3 had received an added side,—two of these by transverse division of a cell beside them, and one by the vertical division of a cell above. Thus they are persistently quadrilateral.

The quadrilateral stomatal cells sometimes arise with great regularity, as shown in the diagrams figure 9, *A* and *B* with actual cells at *C* for comparison.[17] The lowest quadrilateral in column *b* of *C* has grown to the usual extent and probably would not produce a stoma: the four others, shaded, are typical *Mutterzellen*. Nägeli long ago noted that the mother cells remain small while the others grow.[18] The accelerated transverse division in the stomatal columns is demonstrated by counting cells in columns of the same length. For 20 cells in the stomatal column there are 15 in the column beside it. For five divisions in column *b* of figure 9, *C*, there have been only two in the columns on either side, the divisions necessary to produce exactly the pattern in *C* being indicated by dotted lines in *B*. Correlated with retarded cell growth and accelerated division characteristic of the stomatal columns, is the development of the subepithelial air-spaces, the extent of which is shown in the reconstruction, figure 10. As

[16] Thompson, D'A. W. Growth and Form, 1917, p. 395.

[17] Strasburger, in the leaves of iris and hyacinth, finds that the quadrilaterals are cut off invariably (*ausnahmlos*) from the anterior or upper end of the elongate epidermal cells. This may be true of Tradescantia, but there is nothing in figure 9 to indicate from which end they come. Unless some cell has produced quadrilaterals at both ends, they have all come from the same end, however.

[18] Botanische Beiträge. Linnaea, 1842, Bd. 16, p. 238.

the intercellular spaces beneath most of the columns become small (*a*) or are obliterated by collenchymal thickenings (*d*), those beneath the stomatal columns (*b* and *c*) enlarge and form lacunar dilatations under the mother cells. Since intercellular spaces develop most readily at the corners of the cells, the approximation of corners in the small quadrilateral cells may be favorable for the coalescence of these spaces.

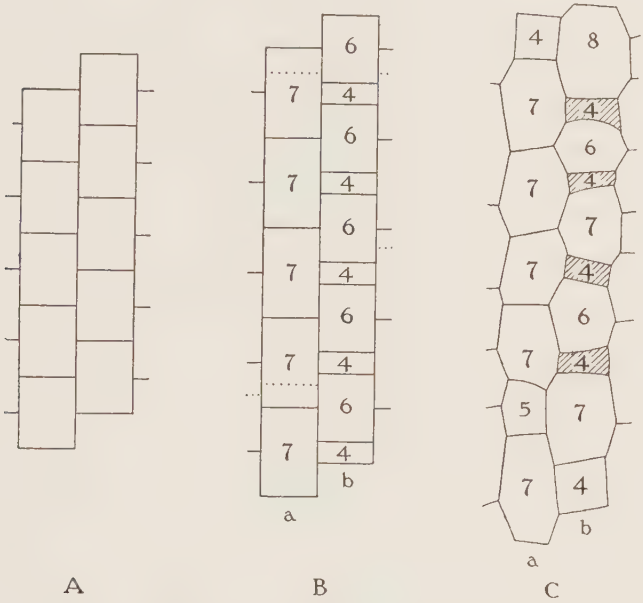

Fig. 9 *A* and *B*, diagrams illustrating the development of stomatal 'mother cells.' *C*, actual pattern from a tangential section of young stem of Tradescantia. × 375 diam.

Not every quadrilateral has a lacunar space beneath it, however.

In the succeeding stage (fig. 11, *A*) the mother cell has increased in volume from 6200 cu.μ to 7800 cu.μ. It contains a nucleus apparently of the same size as those in the general epithelium, which means that its nucleo-cytoplasmic ratio is as high as 1 : 8, whereas that in the general epithelium is perhaps 1 : 40. The lacunar air space, indicated by the stippled

area, has spread beneath the surrounding cells. On either side of the mother cell, a curved vertical wall cuts off a 'lateral accessory guard cell,' having a volume of 2850 cu.μ. It is derived by ordinary mitosis from an epithelial cell in the

Fig. 10 Subepithelial air-spaces beneath two stomatal columns (*b* and *c*) in the immature stem of Tradescantia. Reconstruction from transverse sections. × 400 diam.

adjoining column, which may have a volume of 36,000 cu.μ. Less than 8 per cent. of the volume of these lateral cells is cut off to form the accessory cell, which, since it contains a nucleus of full size, has a nucleo-cytoplasmic ratio of perhaps 1:3. The relation of these cells to the underlying air-space is shown in vertical section in figure 11, *B*.

In the final stage the small quadrilateral mother cell divides vertically into the pair of guard cells, which grow to a volume of 16,500 cu.μ each. Elsewhere in the epithelium quadrilateral cells do not divide, even though they are larger; here they divide invariably. The guard cells have nuclei of normal size, but the stunted growth of the cytoplasm appears in

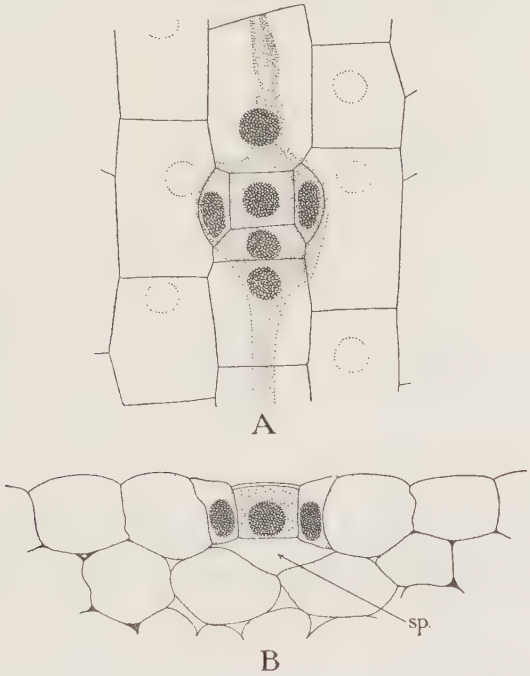

Fig. 11 *A*, subepithelial air-space beneath a developing stoma; reconstruction from transverse sections. *B*, one of the sections, showing the relation of the mother cell and the lateral accessory guard cells to the air-space, *sp*. × 400 diam.

every direction. The cells are shorter, narrower, and thinner than those of the general epithelium. Deficiency in the last of these dimensions is strikingly shown in the occasional stomata in the columnar epithelium of the cucumber, in which the guard cells are one-third the height of the epithelial cells beside them, and fail considerably to reach either the level of the top plate or of the basement membrane.

The lateral accessory guard cells grow from an initial volume of 2850 cu.μ to 25,500 cu.μ, which is as great a growth on its small scale as that of the general epithelium. As a rule these cells remain quadrilateral and undivided. A single instance was observed of the vertical division of one of the accessory guard cells; and in a few instances, after the accessory cell had been made pentagonal by the division of an adjacent cell, it had divided transversely. Figure 11, *A*,

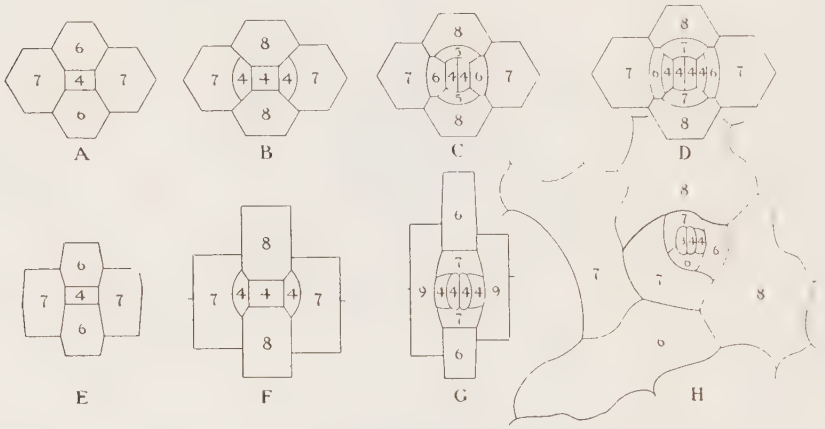

Fig. 12 *A–D*, development of a stoma in *Commelina communis* (after Strasburger). *E–G*, corresponding development in *Tradescantia virginiana*. *H*, a pattern from *Sedum spectabile* (the four peripheral cells reduced in area as compared with the central group). In all these patterns the average number of sides is 6.

shows a small accessory cell cut off below the stoma. Often there is a similar cell above it to complete the pattern, the development of which in Tradescantia has been familiar since Gabreau's description in 1854.[19] As seen in figure 4, all the epithelial and collenchymal cells around the stoma tend to be smaller than elsewhere.

Exceptional in so many respects, the development of the stomatal patterns conforms to the inexorable geometry of

[19] Mémoire sur la formation des stomates dan l'épiderme des feuilles de l'éphémère des jardins, et sur l'évolution des cellules qui les avoisent. Ann. des sci. nat., 4me Sér., Botanique, T. 1, Paris, 1854, p. 213–219.

what has been called Graustein's theorem,[20] of which they afford very simple illustrations. Since these patterns develop by replacement of hexagons, or of polygons having an average of six sides, and since the division planes avoid 4-rayed vertices, the average number of sides of all the groups shown in figure 12 is precisely 6. The development of a stoma in Tradescantia is summarized in *E–G*. *A–D* are based upon Strasburger's figures for the related *Commelina communis* of which he says,—"Hier wird die Complication am höchsten."[21] Stage *C* of Commelina corresponds with *G* of Tradescantia, cell for cell, and the different proportions seem correlated with the isodiametric pattern of the former and the elongate rectangular groundwork of the other. In Commelina each lateral accessory guard cell divides vertically (stage *D*). In a single instance in Tradescantia such a division was seen, but in only one member of the pair of accessory guard cells. Its surroundings were such as to present exactly the arrangement in *D* at one corner of the pattern: at the other end, the transverse accessory cell was of the usual width and extended clear across the lateral accessory cell. In the familiar pattern in Sedum (*H*) *3-sided* cells divide repeatedly, and the mother cell is the last of the group to form, instead of the first. But since three walls meet at every vertex and the group arises in a mosaic of hexagons, the average number of sides of the cells is precisely six.

Relation of the epidermal cells in Tradescantia to the tetrakaidecahedral shape.—Seven years ago the author published his conclusion that cells of approximately the same volume, when occurring in masses, are, on the average, tetrakaidecahedral.[22] The first confirmation by other writers is perhaps that of Hein, who has obtained from mushroom

[20] The facts as to the cells having been observed, including exceptions to Wetzel's theorem, Professor Graustein, at the author's request, formulated the correct substitute to which reference is made. Anat. Rec., 1928, vol. 38, p. 345–346.

[21] Strasburger, E. Ein Beitrag zur Entwicklungsgeschichte der Spaltöffnungen. Jahrb. f. wiss. Bot., 1867, Bd. V, p. 333.

[22] Proc. Amer. Acad., vol. 58, no. 15, June, 1923.

beds a sclerotium in which the cells become massed in a pseudoparenchyma. "It is obvious," he writes, "that the cells just described have an average of six sides in every plane, and have, therefore, an average of fourteen faces."[23] Since such diverse cells as those of this fungus, elder pith, and human fat cells are alike tetrakaidecahedral, it may be assumed, in the absence of evidence to the contrary, that this is the basic shape for all cells in aggregates. The derivation of the epithelial prisms in Tradescantia from tetrakaidecahedra is demonstrated as follows.[24]

In figure 13, *A*, is shown the tetrakaidecahedron in parenchymal orientation, with a surface, and not an edge, above and below. In *B*, a tetrakaidecahedron, similarly oriented, has been drawn as a prism, with all sides of its polygons, as in *A*, of equal length. It is now far from a minimal surface pattern, but shows a uniform and maximum avoidance of 4-rayed vertices. Very significant is the alternating height of the transverse cell walls which meet it laterally—first, one-third of the distance from the top to the bottom, then two-thirds, one-third, etc., as one passes around the prism. If a transverse wall meets the prism two-thirds of the way down, the transverse walls on two adjacent vertical faces and on the vertical face on the opposite side of the prism, are, all three, one-third down.

When a cell like *B* is in a simple epithelium, and so is not surrounded on all sides by other similar cells, four facets are replaced by the free surface: the epithelial cell then has eleven surfaces (fig. 13, *C*). In front view this cell is drawn as *b* in figure 13, *D*, with the epithelial cells *a* and *c* to show the levels of the transverse walls which meet it laterally. The actual levels of these walls for 100 cells have been plotted in figure 13, *E*, which represents a cell in front view (like *b* in *D*)

[23] Hein, Illo. The tetrakaidecahedron in pseudoparenchyma. Bull. Torrey Bot. Club, 1930, vol. 57, p. 59–62.

[24] It may be inferred that the cambial cells have not yet been adequately studied, and that the simple models of them often seen in botanical laboratories need revision. They are presumably derived from 14-hedra in the prosenchymal orientation.

divided into tenths. The graph on the right indicates that on that side no transverse wall met the 100 cells in the upper tenth of their length; there was one wall in the second tenth; ten in the third, and so on. The alternation between the two-thirds and one-third levels on the opposite sides of the cell is

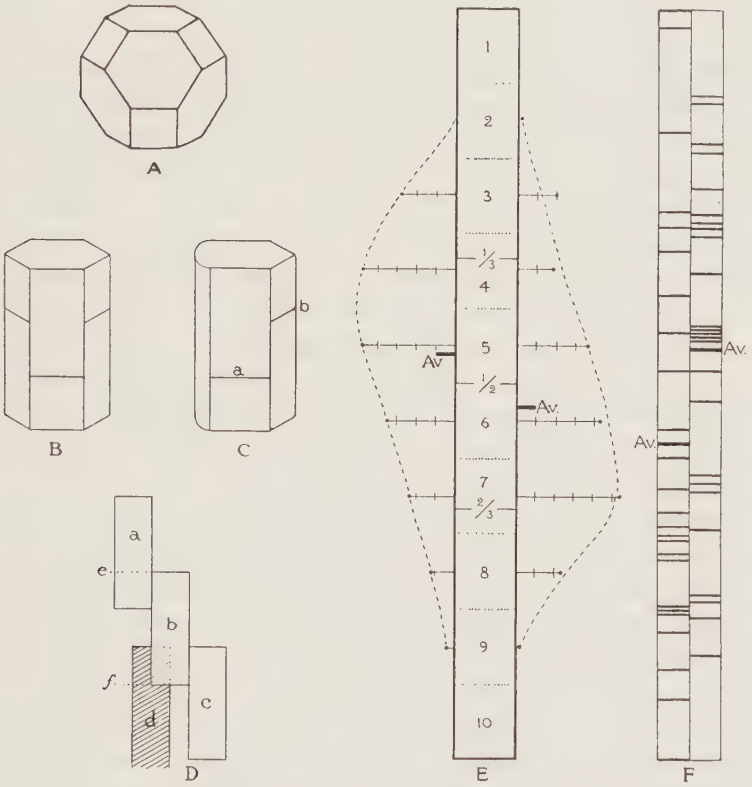

Fig. 13 *A*, orthic tetrakaidecahedron in parenchymal orientation. *B*, prismatic isolateral tetrakaidecahedron, oriented as in *A*. *C*, epithelial form of *B*, having 11 surfaces. *D*, relation of epithelial cells, having the form of *C*, to one another as seen in front or surface view. *a*, *b*, *c*, epithelial cells; *d*, an underlying cell. *E*, diagram of a front view of a hexagonal epithelial cell in a mature Tradescantia stem. The cell is divided into tenths; and the levels at which 100 transverse walls met such a cell laterally have been plotted on either side. *F*, diagram of the cell shown in *E*, also seen from in front, with the levels at which 50 transverse walls between collenchymal cells met its basal surface,—25 on either side of the median line. The median line is the projection of the median ridge of the wedge-shaped basal surface.

clearly indicated in the graphs, but there is a marked trend toward the halfway position which would bring the transverse walls opposite each other. Eight cells had both walls in the middle tenth: 37 cells had both walls in either the upper or lower half of the cell. Accordingly the average levels on the two sides (fig. 13, *E, Av*) are closer together than in the norm.

When the hypothetical cell *C* of figure 13 is in contact with underlying cells of the same length as itself, it is in relation with four of them, and their transverse walls are at the same two levels as the transverse epithelial walls, but alternating with them (*cf. b.* in fig. 13, *C,* and the top of the underlying cell *d* in *D*). If, however, the underlying cells are longer than the epithelial cells, some of the transverse walls will be eliminated. This is the case in Tradescantia.

It is possible that the collenchymal and epithelial cells in Tradescantia are primarily of the same length, since toward the lower node I have seen a small area in which a few collenchymal and epithelial cells averaged 93 and 96 μ in length, respectively. But if the collenchymal cells divide transversely less frequently than the epithelial cells, they inevitably become longer, and a gradually shifting pattern takes place. If the collenchymal cells are twice the length of the epithelial cells, each of the latter is in contact, on the average, with three underlying cells instead of four. In a mature stem of Tradescantia, 50 collenchymal cells were measured by counting the 15-μ sections into which they had been cut, and they averaged 1067 μ or 3.1 times the length of the epithelial cells of the same region. The relative lengths are probably not simple multiples, and if they start as equal, must pass through all fractional gradations; but in this specimen the collenchymal cells are close to 3 times the length of the epithelial. In that case a collenchymal cell should have, on the average, contacts with 8 epithelial cells, and the group measured for length actually averaged 7.8 contacts. Consequently, instead of being in relation with four collenchymal cells, an epithelial cell has an average relationship with two

and two-thirds. Many epithelial cells have no transverse wall crossing their basal surface, and so have nine facets altogether. Others have one such septum and ten facets: still others have two septa and eleven facets, the average being nine and two-thirds when the collenchymal cells are three times the length of the epithelial.

The levels of the transverse walls between collenchymal cells, under these conditions, might be expected to have a random arrangement in relation with the epithelial cells, but this is not the case. The rectangular outline of figure 13, *F*, represents the basal surface of an epithelial cell seen from in front, being oriented precisely as in *E*. It is bisected vertically by the projection of the median ridge of its wedge-shaped base. The levels of 50 transverse walls between collenchymal cells, 25 on either side, are indicated by transverse lines, the observations being made on the same group of cells which yielded figure 13, *E*. The average levels, *Av.*, not only alternate with each other, but with those of the transverse epithelial contacts shown in *E*. The intervals are not thirds; but otherwise all the features of this peculiar pattern are those presented by the hypothetical cell *C*,—an epithelial cell on the tetrakaidecahedral plan.

Summary.—Comparing the 60-mm. and 220-mm. cucumbers, it appears that the growth of the epithelium is from 24 cu.mm. to 1700 cu.mm., or to 70 times its initial volume. This gives no information about the growth or shape of the epithelial cells, which are dependent respectively on the number of cell divisions and on the planes of cell division. Measurements show that the growth of the average epithelial cell in these cucumbers is from 520 cu.μ to 7280 cu.μ, or to 14 times its initial volume. The number of cells increases from 46,250,000 to 234,000,000, or 5 times. Five times as many cells, 14 times as large, accounts for the 70-fold growth of the epithelium as a whole. Five times as many cells is equivalent to the division of every cell, the division of every daughter cell, and of one-fourth of the grand-daughter generation. If no cell in the third generation had divided, the cells

would be 25 per cent. larger—they would have grown 25 per cent. more: and if all cells in the third generation had divided, the cells would have grown to only five-eighths of the size actually attained. Clearly the growth of the cells depends on two factors,—the growth of the tissue as a whole and the infrequency of cell division.

The shape of the cells depends on the planes of division. The cells of the cucumber are tall radial columns since no tangential divisions occur to reduce the radial dimension. They are isodiametric on surface view since elongation in any direction in that plane is equally subject to reduction by division. Should transverse divisions be relatively infrequent or fail to occur, the cells of the cucumber would become long lengthwise of the stem as in Tradescantia. With the same growth of the tissue and a sufficient number of transverse divisions the cells of Tradescantia would have long axes *across* the stem. Clearly the shape of the cell depends on the planes of division, and these planes are rigorously determined.

Whenever a cell in an epithelial mosaic divides perpendicularly to the free surface, six sides are added to the mosaic. Four-rayed intersections being avoided, the average number of sides remains precisely six, in accordance with Graustein's theorem. Patterns controlled by this theorem are seen in epithelia everywhere, but very handsomely in the stomatal groupings. Just as surely as the average number of sides is six in surface view, it is six also in vertical sections of massed cells, and the total number of surfaces per cell averages fourteen. The derivation of the prismatic epithelial cells of Tradescantia from the 14-hedral form to shapes averaging nine and two-thirds surfaces has been shown. The shape of the cell, instead of being its most variable and inconsequential feature, may come to be that which is best understood and accounted for.

Associated with the development of subepithelial air-spaces, cell division is stimulated, so that small quadrilateral and triangular cells divide, and cell growth is retarded. Minot's

laws of senescence are here reversed, since cells which cease to divide, and in case of the guard cells become highly differentiated, have large nuclei and little cytoplasm. Apart from the stomata, in the general epithelium, the growth of a cell depends on its number of sides or surfaces. Consequently, growth which follows division is manifest as much in two adjacent cells (which receive added sides) as in the daughter cells. This appears in the tabulation for Tradescantia, in striking accord with the previous tabulation for Cucumis. The modus operandi seems to be that the new sides introduced by division are predominantly short ones, and there follows a readjustment and lengthening of such sides to equal those in the rest of the tissue. This means a special growth of certain cells after division. The cells with an excess of sides due to the division of those beside them, enlarge above their fellows, and are then reduced by division. It appears to be a physical rather than a chemical process, and is therefore less likely to be understood by observing effects of toxic solutions and fertilizers, than by a morphological study of emulsions.

THE EFFECTS OF THYMECTOMY ON YOUNG FOWLS

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ONE FIGURE

INTRODUCTION

The thymus is an organ of which much has been suspected but little is known. It is prominent in young animals, but its normal involution usually begins at sexual maturity and continues with varying rapidity throughout life. Involution of the lymph glands that it so much resembles also begins at sexual maturity. The major portion of the thymus nearly always arises from endodermal epithelium and is thus related to pharyngeal derivatives like the thyroid and parathyroid glands. It becomes infiltrated with cells resembling lymphocytes and takes on a lymphoid appearance. Whether its function is lymphoid, as seems likely, or whether it may be endocrine is not known. That the thymus may have an endocrine function and that it may be connected with calcium metabolism in birds has been suggested more than once. If it has a specific function, it seems that its removal as completely as possible from chicks and young fowls might suggest the nature of its influence; hence the experiments described here are made.[1]

Literature relating to the thymus gland has been surveyed by Park and McClure ('19), Hammar ('21), Crotti ('22), and Marine ('28). Only brief mention of a few of the more important investigations and those concerned with birds is

[1] The authors are indebted to Mr. Lovell Smith, of South Hadley, Massachusetts, for help in caring for the fowls as well as for assistance in making records of body weights and eggs and for suggestions during the progress of the experiments.

made here. The literature of the experimental work on the thymus shows that both thymectomy and feeding thymus have given contradictory or unsatisfactory results in amphibians, birds, and mammals. Few experiments have been done on fish and reptiles. In the main, two opinions have been held: that the thymus is a gland of internal secretion, and that it is a lymphoid organ connected with phases of metabolism, but without endocrine importance. Among the investigations that have given some support to the first contention are those of Gudernatsch ('12) and others, who reported that feeding thymus to tadpoles delayed the metamorphosis; Soli ('09), who concluded that it played a part in calcium metabolism, finding that thymectomized hens laid small eggs with imperfectly calcified shells; and Riddle ('24), who was able to remedy shell deficiencies of doves and pigeons by giving daily doses of thymus. Other workers have discovered no endocrine function in the organ. Swingle ('17) fed thymus to tadpoles and discovered no resulting symptoms; and, among others who worked on mammals, Van Allen ('26) performed thymectomies on rabbits, and Park and McClure ('19), on dogs, without recognizing symptoms attributable to endocrine disturbance.

Among conflicting reports of thymus experiments, the repeated appearance of certain results shows them to be well founded. Although complete thymectomy is difficult, it has been clearly demonstrated in frog tadpoles (Allen, '18), rats (Pappenheimer, '14), and some other animals. The thymus is not essential to life and its removal has not been attended by any constant symptoms thus far discoverable. Regeneration of remnants of the thymus proceeds regularly after the major part of it has been removed (Gottesman and Jaffe, '26). The thymus appears to be in some way interrelated with the sex glands and the suprarenals (Henderson, '04; Marine, Manley, and Baumann, '24).

Results of investigation upon the thymus of birds are confused and inconclusive. Tarulli and Monaco ('97) operated on eighteen chickens, the only two that survived thymectomy

being normal in twelve days; a second lot thymectomized after twenty-five days of age showed no symptoms. In 1908, Basch removed all the visible thymus from two pigeons four weeks old. When they were killed six and eight weeks later, he found as much thymus tissue present in them as in their controls. He repeated the experiment in five young chicks and in about three months found a similar regeneration.

In two later investigations Soli ('09) and Riddle ('24) reported a connection between the thymus and egg production in fowls and pigeons. Soli thymectomized four laying hens, which laid eggs with normal shells until seventeen to twenty days after the operation and then began to produce eggs with defective shells, continuing to do so until twenty-eight to forty days after the operation, when they again laid eggs with normal shells. Among large numbers of doves and pigeons being kept under observation, Riddle at first noted that certain females showed various reproductive disorders, such as asymmetrical and small eggs, small yolks, deficient egg envelopes, single clutches, and infertility. Among such birds were some whose eggs were consistently deficient in albumen and shell; when such birds were killed and examined, they were found to have extremely small thymi. Riddle fed five pigeons of this type with daily doses of desiccated ox thymus; after such treatment, they began to lay eggs with normal envelopes. Thymus was the only substance that restored their capacity to secrete normal albumen and shell, although other tissues, foods, and endocrine substances were administered. Riddle concluded that insufficient thymus was associated with deficient secretion of the oviduct. He then attempted to find whether either partial or complete thymectomy would correlate with deficient egg envelopes, as the small thymi had seemed to do. Of the five birds that he thymectomized, only one survived to produce eggs. In fifteen days after the operation and for the following two months, this pigeon laid eggs with noticeably soft shells, but after that time the shells became normal again. From the foregoing data and from the results of Soli ('09), Riddle ('24) con-

cluded that the thymus must have a specific action on the oviduct of birds.

Kříženecky ('29) showed that the decrease of body weight produced by feeding dry thyroid to pigeons could be reduced one-half by simultaneously feeding with dry thymus. He interpreted this result as a hormonal antagonism between the thyroid and thymus.

In December, 1929, Ackert and Morris and Morgan and Grierson simultaneously published abstracts that agreed in main points regarding thymectomized chicks and young fowls. Ackert and Morris ('29) detected no constant differences between the thymectomized and control birds as to weight of eggs, strength and appearance and thickness of eggshells, calcium carbonate in eggshells, fertility and hatchability of eggs, as well as in the weights of certain organs and the haemoglobin content of blood. Morgan and Grierson ('29) also reported that in a series of thymectomized fowls the size of eggs and the amount of albumen and the shell were entirely normal. The following account presents the details of their experiments.

MATERIAL AND METHODS

The fowls used in these experiments were Rhode Island Reds (series B) hatched on November 11, 1928. A few Plymouth Rocks (series A) hatched on October 9, 1928, were used for the weights given in table 1. Operations and observations were carried on during the fall of 1928 and the spring of 1929 and a few in July, 1930. The majority of the evidence here presented rests upon seven controls (three male, four female) and twenty thymectomized birds (eleven male, nine female), called series B. Most of these individuals had two operations, some had three, and one a fourth operation (table 2).

From soon after hatching until February, the chicks were confined in sunlit rooms with sanded floors; they then were transferred to a poultry house with an open runway. For the first few weeks they were fed chick-mash mixed with a daily

ration of ten to twenty drops of cod-liver oil and a sprinkling of scratch-feed; milk and chopped lettuce were also supplied to them. In February, laying-mash and scratch-feed without cod-liver oil were substituted for the other mixture. In April, cracked oyster shells were added, in order to provide an adequate calcium allowance. Each chick wore a numbered leg band and individual records were kept on cards on which weights, descriptions of operations, appearance, and further data were written.

These experiments consisted of the removal of the thymus as completely as possible from healthy chicks of known age and weight, and subsequent observations of their body weight and appearance, feathering, production of eggs and egg envelopes, together with repeated operations to remove regenerating thymus.

Thymus was removed from only the healthiest individuals. The bird was kept without food during the part of the day preceding the operation and weighed just before it. When it was etherized, its head was placed on a cotton pad, with one side of the neck strongly illuminated and the feathers dampened and laid back. An incision was made, the wound moistened with sterile salt solution, and the thymus tissue then gently pulled out of the surrounding fat and away from the closely associated jugular vein and carotid artery. Thymus tissue extends in a series of irregular lobes on each side of the neck along the jugular veins and down past the clavicles toward the thyroid gland near the thoracic cavity. After all visible thymus had been removed, the skin was sutured and the same operation repeated on the other side of the neck. Care was taken to keep the operated animals thoroughly warm, and within an hour they were usually entirely recovered. In two or three hours they were fed and then allowed to run with the other chicks. The freshly extirpated thymic tissue was weighed and preserved in Bouin's fluid for sectioning.

From the first operation on through the first molt during January (1928) up to June (1929), records were kept of changes in appearance, such as size, feathering and sex char-

acters. From April 8, 1928, when the first pullet laid, to May 9, 1928, descriptive records were kept of all the eggs that could be definitely ascribed to individuals. These eggs were first weighed whole, then the yolk and shell weights were taken separately; shells were always examined for thickness or thinness. No attempt was made to measure their thinness by the rate of loss of water through them (Riddle, '24), this seeming to be a test of porousness rather than of thickness. As already stated, operations were made two, three, or four times on thymectomized fowls in order to remove any remaining thymus tissue. Exploratory operations were also made upon controls in order to note the condition of the normal thymus.

TABLE 1

Comparison of weights of body and freshly extirpated thymus in series A, five chicks, thirty-two days of age

NO.	WEIGHT OF BODY, GRAMS	WEIGHT OF THYMUS, GRAMS	PER CENT OF THYMUS TO BODY WEIGHT
A 3 female	112	0.233	.21
A12 male	118	0.270	.23
A18 male	104	0.255	.25
A24 female	99	0.185	.19
A25 female	122	0.270	.24

EXPERIMENTS AND RESULTS

Before the experiments were undertaken, dissections of thirteen- and fourteen-day chicks were made to determine the normal extent of their thymic tissue. First the entire chick and then its extirpated thymus were weighed in order to calculate the proportion of thymus to body weight. This was repeated upon five chicks at eighteen days of age (thymus weighed after fixing in Bouin's fluid) and upon five more at thirty-two days of age (table 1, fresh thymus weighed at removal).

The thymus was removed from a selected group of Rhode Island Reds, series B. All the visible thymic tissue was taken out at the first operations, after which growth and general appearance were observed for periods of two to three months,

when a second extirpation was performed on each fowl. Thymectomies were performed on series B primarily to secure females with little or no thymus, but when the operations were first performed it was impossible to tell the sex of the chicks, and weight records were kept for all the birds operated. The whole of series B consisted of seven controls (three male, four female) and twenty thymectomized birds (eleven male, nine female). Table 2 includes the records of thymectomies performed on this series, with weights of body, extirpated thymus, and percentage of extirpated-thymus weight to body weight. Individual records can be followed, except for a group of four male birds and one pullet whose leg bands became broken some time after their first operation. These were renumbered B92 female, B93 male, B94 male, B95 male, and B97 male and included in this table along with the other first-operation birds, though individual weights could not be given for them until the second operation.

All the fowls mentioned in table 2, although having little or no thymus, lived and grew as normally as the controls. A graph (fig. 1) showing growth that occurred between three and 160 days of age in seven controls and in the twenty operated birds shows a similarly steady upward trend with the controls only very slightly higher. This difference between the points on the curves at 130 days, for example, was found to be less than its probable error and therefore not significant.

The thymectomized birds appeared in every way as healthy as the controls. Feathering also was entirely normal in the experimental animals. Regeneration of the thymus occurred, notably in cases B32 (female), B49 and B97 (male). All of the thymus tissue that could be found by careful scrutiny was removed. The small lobules that escaped such examinations sometimes increased considerably in size, thymus removed at second or third examination being easily recognizable. The pieces of extirpated thymus were sectioned and examined histologically.

While the data in table 2 indicate that complete extirpation is difficult, nevertheless the histories of B59, B60, B61, B91,

TABLE 2

Records of thymectomies performed on individuals of series B, with weights of body and extirpated thymus and per cent of extirpated thymus to body weight

DATE	NO.	AGE	WEIGHT OF BODY, GRAMS	WEIGHT OF THYMUS, GRAMS	PER CENT OF THYMUS WEIGHT TO BODY WEIGHT
			First operation		
12/ 8/28	B32 ♀	27	130	0.258	0.19
12/ 8/28	B60 ♀	27	140	0.455	0.33
12/13/28	B54 ♂	32	200	0.862	0.43
12/13/28	B80 ♂	32	163	0.530	0.33
12/15/28	B62 ♀	34	185	0.714	0.39
12/15/28	B66 ♂	34	163	0.645	0.40
1/31/29	B35 ♀	81	1060	2.975	0.28
1/31/29	B38 ♀	81	1010	5.370	0.53
1/31/29	B50 ♂	81	1260	4.190	0.33
1/31/29	B56 ♂	81	1270	5.230	0.41
1/31/29	B91 ♂	81	1215	4.790	0.39
2/13/29	B61 ♀	94	1080	6.320	0.59
2/13/29	B96 ♀	94	1150	2.400	0.21
2/16/29	B59 ♀	97	1470	7.110	0.48
2/16/29	B74 ♀	97	1330	5.215	0.39
2/22/29	B70 ♂	103	1980	6.080	0.31
	B92 ♀	Renumbered 1/10/29			
	B93 ♂				
	B94 ♂				
	B95 ♂				
	B97 ♂				
				Average,	0.375
			Second operation		
2/ 1/29	B32 ♀	82	1040	1.055	0.10
2/ 1/29	B60 ♀	82	1240	0.815	0.07
2/ 9/29	B54 ♂	90	1500	0.180	0.01[2]
2/16/29	B80 ♂	97	1820	0.545	0.03[2]
4/20/29	B62 ♀	160	2240	0.056	0.003[2]
2/ 9/29	B66 ♂	90	1385	0.075	0.005[2]
4/10/29	B35 ♀	150	1920	1.172	0.61 (d 4/14)
4/20/29	B38 ♀	160	2290	0.085	0.004[2]
[1]	B50 ♂				
[1]	B56 ♂				
2/ 9/29	B91 ♂	90	1230	None	0.0[2]
4/20/29	B61 ♀	160	2140	None	0.0 (d 4/25)
[1]	B96 ♀				
4/10/29	B59 ♀	150	2350	0.051	0.002
4/24/29	B74 ♀	158	....	None removed[2]	
[1]	B70 ♂				
4/10/29	B92 ♀	150	2420	0.337	0.01
2/23/29	B93 ♂	104	1710	0.180	0.01[2]
2/23/29	B94 ♂	104	1760	0.160	0.01[2]
2/23/29	B95 ♂	104	1670	0.553	0.03[2]
2/ 9/29	B97 ♂	90	1100	0.310	0.03[2]
				Average,	0.03
			Third operation		
4/ 6/29	B32 ♀	146	2100	0.073	0.003
4/ 6/29	B60 ♀	146	2200	0.016	0.0007
5/ 7/30	B59 ♀	542	....	0.0	0.0
5/ 6/30	B92 ♀	541	....	0.0	0.0
			Fourth operation		
5/ 2/30	B60 ♀	537	....	0.0	0.0

[1] Killed without second operation because of inadequate facilities for keeping.

[2] Killed without third operation because of inadequate facilities for keeping.

d = died.

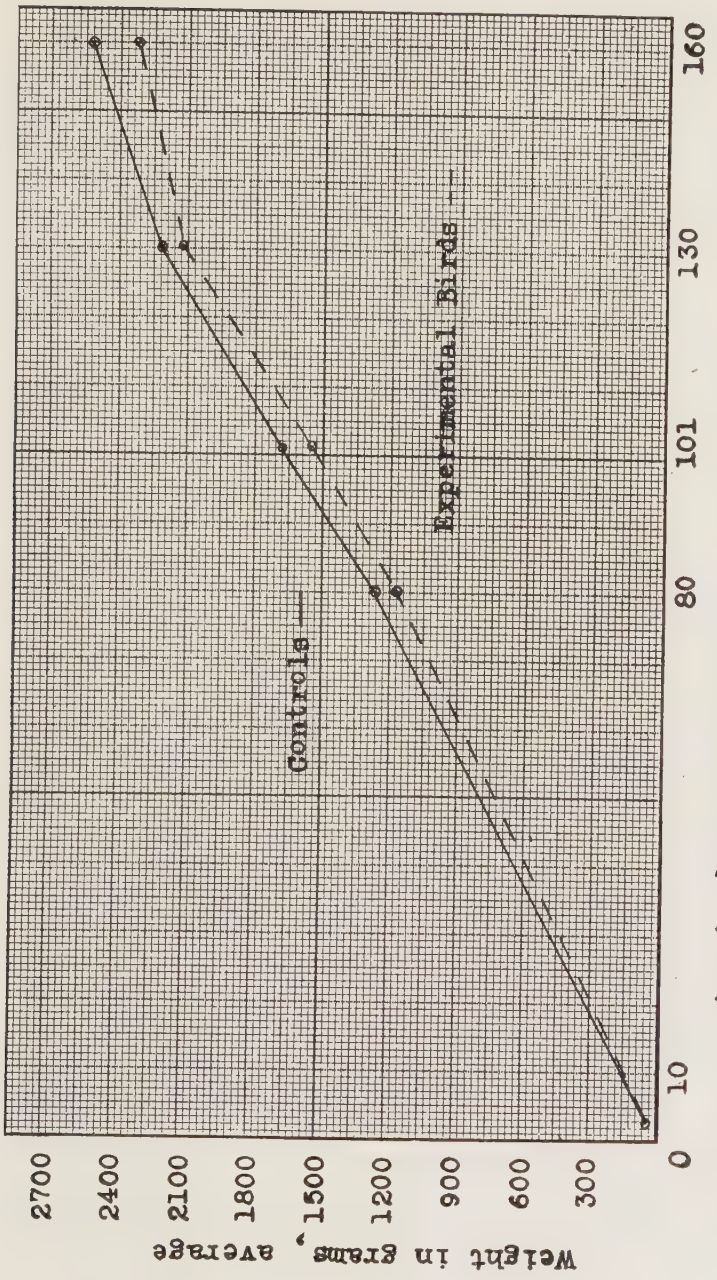

Fig. 1 Increase of weight between third and 160th days of age; above, growth of seven controls; below, growth of twenty thymectomized fowls. Numbers at left indicate the average of weights of fowls at the age indicated below from left to right.

B92 (female) make it seem evident that it is possible to remove it completely in birds as in other types of animals.

It was at first thought that the complete absence of thymus in some operated fowls—B59, B60, B92, B98[2] (female) (table 2)—after a year of observation might be due to the involution of their thymi. This was shown to be improbable, however, when examination of B39, B73, B83 (female), controls of the same age, showed that their thymi had involuted little, if at all. In such control animals nineteen months of age, the thymi were still prominent; thymi removed on July 7, 1930, from control B73 weighed 0.930 gram.

Feathering and the appearance of sex characters were carefully watched in the thymectomized birds. When about three and one-half months old, all the young fowls began to develop combs and wattles, but there were no significant differences between those of the control and operated birds nor any noticeable irregularity in other sex characters. Likewise, controls and experimental fowls went through their first molts in similar fashion.

Records of the eggs of individuals of series B were kept, in order to find out whether egg production had been reduced and whether the eggs were smaller or the egg envelopes deficient after thymectomy, as was observed by Riddle ('24). The eggs were weighed on the day they were laid, the whole egg, then the yolk taken separately; when this could not be done on the same day with laying, the weights were not recorded. Table 3 includes the histories of egg production in four controls and six thymectomized birds. The other three thymectomized pullets did not lay until after May 9, 1929. Deficient albumen and shells were looked for, especially following the period beginning seventeen to twenty days after the operations, but no defects in any way unusual appeared either in this period or in the one immediately following the operations.

[2] B98 was a thymectomized fowl renumbered in May, 1930, whose record could not therefore be included.

Pullet B59, first thymectomized when ninety-seven days old, laid a normal egg two days before the second operation on April 10th, when 150 days old, and again an entirely normal egg on April 15th and on succeeding dates (p. 112). Pullet B32, first thymectomized when thirty-two days old, laid a normal egg on April 10th, four days after a third operation performed when she was 146 days old. The shell of this first egg was thin, but by no means soft or flexible; shells of the succeeding ones were completely normal (table 3). Almost complete lack of thymus evidently did not retard the egg production in these birds, since thymectomized pullets B59 and B32 laid before the controls, B39 and B83, of the same age. That the controls must have possessed a good amount of thymus at this age of 148 days was shown by the subsequent examination of them in May, 1930 (about 540 days old), when abundant thymus was found in them.

There appeared to be no significant difference between the eggs of controls and of thymectomized pullets. The controls generally varied slightly less in total egg weights and in yolk weights than did the thymectomized birds. In the interval of twenty to twenty-five days after the third operation B32 showed an increase in the quantity of egg envelopes, but B59 showed a decrease. Pullets B60 and B92 did not lay during the three weeks following their operations, but in the period following that, the time emphasized as crucial by Riddle ('24), they produced eggs with normal albumen and shell.

Weights of egg envelopes (albumen, membranes, and shell) secreted by controls and operated pullets were also compared and per cents of yolk weight and of egg-envelope weight to that of the whole egg were figured. The results for four thymectomized birds (B59, B92 had been operated twice and B32, B60, three times, table 2) and two controls are shown in table 4.

None of the thirty-four eggs of thymectomized fowls in table 4 had soft shells, yet these four birds had little or no thymus during a good part of their lives (table 2). Of a total of 106 eggs laid by nine thymectomized and four control fowls

TABLE 3

Egg production of controls of series B, April 8 to May 9, 1929

	DATE	TOTAL WEIGHT OF EGG	WEIGHT OF YOLK	PER CENT OF YOLK WEIGHT TO TOTAL	REMARKS ON SHELL, ETC.
Control B39	4/20	47.910	9.095	18.9	Shell normal
	4/22	44.450	9.660	21.7	Shell normal
	4/24	Not weighed	....	...	Shell normal
Control B73	4/22	37.710	10.500	27.8	Shell normal
	4/25	Not weighed	....	...	Thin-shelled
	5/1	Not weighed	Large	...	Shell normal
	5/6	38.215	11.222	29.4	Shell normal
	5/8	42.200	11.767	27.8	Shell normal
	5/9	42.533	11.340	26.6	Shell normal
Control B83	4/19	34.465	Broken	...	Shell defective
	4/22	43.690	11.075	25.3	Shell normal
	4/24	Not weighed	....	...	Shell normal
	4/25	Not weighed	....	...	Shell normal
	4/26	Not weighed	....	...	Shell normal
	4/29	44.870	12.780	28.5	Shell normal
	5/2	Not weighed	....	...	Shell normal
	5/3	Not weighed	....	...	Shell normal
	5/6	48.150	12.365	28.5	Shell normal
	5/7	50.910	11.505	22.6	Shell normal
	5/8	63.650	21.410	(33.7)	Double-yolked; normal shell
	5/9	48.425	12.195	25.2	Shell normal
Control B84	4/22	39.175	10.300	26.3	Shell normal
	4/23	42.120	9.610	22.8	Shell normal
	4/24	Not weighed	....	...	Shell normal
	4/27	Not weighed	....	...	Shell normal
	5/1	46.280	11.650	25.2	Shell normal
	5/5	47.990	....	...	Shell normal
	5/7	46.905	12.175	26.0	Strong shell
	5/8	44.405	12.235	27.6	Shell normal
	5/9	47.955	12.003	25.0	Shell normal

Egg production of thymectomized fowls of series B, April 8 to May 9, 1929

	DATE	TOTAL WEIGHT OF EGG	WEIGHT OF YOLK	PER CENT OF YOLK WEIGHT TO TOTAL	REMARKS ON SHELL, ETC.
B32. Thymectomized. First egg laid 4 days after third operation	4/10	32.695	9.175	28.1	Shell thin; not soft
	4/11	34.265	9.685	28.3	Shell normal
	4/12	34.135	10.090	29.6	Shell normal
	4/13	35.270	Broken	...	Shell normal
	4/14	38.450	10.330	26.9	Shell normal
	4/21	39.475	Broken	...	Shell normal
	4/23	36.905	9.735	26.4	Shell normal
	4/24	Not weighed	....	...	Shell normal
	4/29	41.960	11.080	26.4	Shell normal
	5/5	41.180	....	...	Shell normal

TABLE 3—*Continued*

	DATE	TOTAL WEIGHT OF EGG	WEIGHT OF YOLK	PER CENT OF YOLK WEIGHT TO TOTAL	REMARKS ON SHELL, ETC.
1359. Thymectomized. First egg laid	4/8	32.938	8.315	25.2	(First egg laid in flock.) Shell thin; not soft
2 days before the second operation	4/15	42.465	10.130	23.9	Shell normal
	4/22	44.905	10.150	22.6	Imperfect shell; not soft
	4/24	Not weighed	....	...	Shell normal
	4/25	Not weighed	....	...	Shell normal
	4/26	Not weighed	Large	...	Shell normal
	4/29	42.172	11.520	27.3	Shell normal
	5/4	Not weighed	....	...	Shell normal
	5/8	55.010	Broken	...	Shell normal
1360. Thymectomized. First egg laid 19 days after third operation	4/25	Not weighed	....	...	Shell normal
	4/26	Not weighed	....	...	Heavy shell
	4/27	Not weighed	....	...	Shell normal
	5/1	47.625	11.097	23.3	Shell normal
	5/2	Not weighed	....	...	Shell normal
	5/5	56.420	Double	...	Shell normal
	5/6	48.025	Broken	...	Shell normal
	5/7	46.425	10.539	22.7	Strong shell
	5/8	55.660	12.500	22.5	Shell normal
	5/9	49.655	11.770	23.7	Shell normal
1362. First egg laid the day before second operation	4/19	37.795	9.800	25.9	Shell normal
	4/22	Not weighed	....	...	Membranous shell—smashed
1374. First egg laid 3 days before second operation	4/21	47.613	Broken	...	Shell normal
	4/23	41.955	10.310	24.6	Shell normal
	4/24	Not weighed	....	...	Shell normal
1392. Thymectomized. First egg laid 10 days after second operation	4/20	Not weighed	....	...	Shell normal
	4/22	61.265	Broken, double	...	Shell normal
	5/4	Not weighed	....	...	Shell normal
	5/6	44.315	11.770	26.3	Shell normal
	5/9	43.480	12.765	29.4	Shell normal

TABLE 4

Percentage of weight of yolk and yolk envelopes (albumen, membrane, shell) to weight of whole eggs of controls and thymectomized fowls

NUMBER	TOTAL OF EGGS LAID	PER CENT OF YOLK WEIGHT	PER CENT OF ENVELOPE WEIGHT
Controls:			
1383	12	26.1	73.9
1384	9	25.5	74.5
Thymectomized:			
1332	10	26.8	73.2
1359	9	24.7	75.3
1360	10	23.1	76.9
1392	5	27.9	72.1

between April 8th and May 9th, six (5.7 per cent) had shells with roughened, imperfect surfaces; three (2.8 per cent) had not been retained in the oviduct long enough to have any membranes; four (3.8 per cent) were soft-shelled; ninety-three (87.2 per cent) had perfectly formed shells.

In their first litters young fowls very commonly lay soft-shelled eggs, the number of them being very largely dependent upon the amount of calcium supplied in their diet. The number of soft-shelled eggs here, only four in 106, is a rather small number for any normal pullets. But the total number of these eggs was laid by a group of pullets about two-thirds of which possessed little or no thymus. Furthermore, the thymectomized hens that were kept through their second summer, B59, B60, B92, B98 (footnote, p. 110), produced a large number of eggs between May 9, 1929, and July 1, 1930, during which time the observation of their egg production was continued. The eggs were not weighed, but the shells were always examined. Although these thymectomized hens possessed no visible thymus (table 2), no one of them has produced a soft-shelled egg during this entire period; the egg-shells have been uniformly very perfect. Pullet B60 deserves special attention; this fowl began to lay nineteen days after her third operation, when she had been almost devoid of thymus for a long time.

DISCUSSION

The results of these thymectomies show that little or no thymus is essential to the life or to the normal growth and development of the domestic fowl. Complete removal of the bird thymus is difficult, but repeated operations have indicated that four complete thymectomies have been performed (B59, B60, B92, B98 (footnote, p. 110)). Regeneration, but not at a rapid rate, occurred in those fowls in which remnants escaped notice at thymectomy (table 2). This is contrary to the findings of Basch ('13), who removed all the visible thymus from two doves, four weeks old, only to discover that at six and eight weeks the doves had nearly as much thymus as their controls.

Bird thymus has been reported to diminish in size more slowly than that of mammals. These experiments seem to bear out this statement. In three controls examined when the birds were about 540 days old the thymi were nearly as large as that of B39 (female) control when 164 days old.

The effects of thymectomy on external sex characters have received little if any attention. Ackert and Morris ('29) could not detect any effect upon the spurs of fowls. In the present study operated birds exhibited no retardation or irregularities in the development of combs, wattles, or plumage.

Contrary to the observations of Soli ('09) upon fowls and of Riddle ('24) upon doves, deficiency of thymus has had no noticeable effect upon egg production. The egg envelopes, albumen, and shells of thymectomized birds showed no significant difference from those of controls; one thymectomized pullet showed an increase in the egg envelopes (B32, female). Soli operated upon fowls after sexual maturity, while these thymectomies were performed upon young chicks and repeated just before or during the first laying season. If thymectomy could inhibit the secretion of egg envelopes, it would seem most likely to have done so in these cases. But instead of producing soft-shelled eggs, beginning at a definite period of two to three weeks after a thymectomy (the time emphasized by Riddle), these pullets of series B produced normal eggs with an unusually small percentage of soft shells for pullets' eggs. These results agree with those summarized by Ackert and Morris ('29). Judging from the present experiments, it seems unlikely that removal of the thymus can bring about any such definite abnormalities in birds as have previously been reported.

SUMMARY

1. The effects of thymectomy have been studied in twenty young fowls partially or completely thymectomized.

2. The operations were repeated at intervals on sixteen of the total twenty fowls, to search for and remove remaining or regenerated thymus.

3. Complete removal of the thymus in birds is difficult, but is believed to have been achieved in at least four cases.

4. Regeneration of the thymus progressed at a moderate rate.

5. Neither partial nor complete thymectomy appeared to affect normal living and growth, the development of comb, wattles, and feathers, or the progress of the first molt.

6. Partially and completely thymectomized hens produced entirely normal eggs with normal amounts of albumen and shell, both in the interval of two to three weeks directly after the operation and in the time succeeding that period. There was no sign that calcium metabolism was in any way affected by thymectomy. Hens made thymus-free by repeated operations were fine, healthy fowls and continued a high production of perfect eggs during the following year of observation.

7. These experiments have presented no evidence that the thymus has an endocrine function in birds.

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THE EFFECT OF HYPOPHYSECTOMY UPON THE INVOLUTION OF THE THYMUS IN THE RAT[1]

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FOUR FIGURES

The difficulty of determining whether an alteration in any organ after hypophysectomy is due directly to the absence of the pituitary secretions or indirectly to other upsets is even greater with the thymus than with most of the other glands, because of its responsiveness to general body conditions and to alterations in other organs. Some of the glandular changes induced by hypophysectomy in rats would be expected, from experimental work on the thymus reported by other investigators, to retard, and others to hasten, thymic involution or, indeed, to cause an actual hyperplasia. The study was begun with the expectation of finding that the ablation of the hypophysis slowed the involution of the thymus, since one of the striking effects resulting from the loss of the pituitary is a failure of the gonads and other reproductive organs to develop.[2] Instead of a retardation, however, an accelerated rate of involution was found. The factors responsible for this I will discuss later in the paper.

EXPERIMENTAL FINDINGS

As is shown in the table, autopsy data have been secured from seventeen rats hypophysectomized by a method by

[1] Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[2] The disabilities caused by the ablation of the hypophysis in the rat have been described in a recent paper (Smith, '30), and it consequently is unnecessary to present them in detail here.

Table giving ages, general body weights, and total lengths at the beginning and the end of the experiment, together with the weights of the reproductive organs and thymus and the percentage weight of the latter on body weight and the relative percentage value of the thymus with that of reference control as unity

DESIGNATION OF RAT	TYPE	EXPERIMENT BEGUN[1]				EXPERIMENT TERMINATED								
		Age, days	Total weight, grams	Total length, millimeters	Sexually mature	Age, days	Total weight, grams	Total length, millimeters	Number of days after operation	Thymus, grams	Gonads, grams	Sex apparatus, grams	Percentage thymus weight on body weight	Relative per cent thymus, reference control being unity
						Females								
W4020	Reference control	41	104	295	No				0	0.2562	0.0130	0.0346	0.246	1.00
GH4021	Hypophysectomized	41	122	317	No	105	129	322	64	0.1211	0.0031	0.0415	0.094	0.38
W4022	Control	41	96	294	No	105	198	363	64	0.2623	0.0397	0.2145	0.132	0.54
GH2889	Reference control	43	95	292	No				0	0.2670	0.0166	0.0540	0.28	1.00
W2890	Hypophysectomized	43	110	296	Yes	67	108	297	24	0.2354	0.0208	0.0615	0.21	0.75
W2891	Control	43	92	281	Yes	67	141	328	24	0.400	0.0527	0.2985	0.30	1.07
W2913	Reference control	45	98	274	No				0	0.2489	0.0136	0.0450	0.25	1.00
W2911	Hypophysectomized	45	91	275	No	272	72	293	227	0.0206	0.0032	0.0372	0.03	0.12
W3789[2]	Control	45	96	281	Yes	272	248	305	227	0.1134	0.0532	0.4107	0.04	0.18
BH3900	Reference control	45	112	296	No				0	0.2950	0.0163	0.0502	0.26	1.00
BH3901	Hypophysectomized	45	124	297	No	199	111	308	154	0.0742	0.0035	0.0410	0.07	0.27
BH3902	Hypophysectomized	45	135	302	No	200	124	308	155	0.0706	0.0033	0.0494	0.06	0.23
BH3904	Hypophysectomized	45	119	299	No	200	123	302	155	0.0490	0.0028	0.0416	0.04	0.15
BH3903	Control	45	106	289	No	200	226	353	155	0.1957	0.0393	0.426	0.086	0.33
GH3060	Reference control	49	126	308	Yes				0	0.2254	0.0178	0.0746	0.18	1.00
GH3062	Hypophysectomized	49	135	322	Yes	222	103		173	0.0272	0.0137	0.0602	0.02	0.11
GH3063	Hypophysectomized	49	136	317	Yes	255	116	316	206	0.0316	0.0119	0.0658	0.027	0.15
BH3061	Control	49	125	314	Yes	254	247		205	0.1602	0.0625	0.5224	0.065	0.36

BH2992	Reference control	50	137	315	Yes				0	0.3296	0.0162	0.0583	0.24	1.00
W2993	Hypophysectomized	50	141	307	Yes	332	172	313	282	0.0576	0.0129	0.0728	0.03	0.12
W2994	Control	50	138	311	Yes	332	311	384	282	0.0940	0.0588	0.5866	0.03	0.12
GH4187[2]	Reference control	61	130	311	Yes				0	0.2145	0.0303	0.1291	0.16	1.00
BH4097	Hypophysectomized	61	109	286	Yes	68	71		7	0.0788	0.0097	0.0632	0.11	0.62
GH4089	Control	61	110	284	Yes	68	110		7	0.3217	0.0182	0.0579	0.29	1.81
BH4075[2]	Reference control	93	147	316	Yes				0	0.2095	0.0226	0.2453	0.14	1.00
BH2827	Hypophysectomized	93	155	347	Yes	329	110	357	236	0.0217	0.0056	0.0702	0.02	0.14
BH2825	Control	93	148		Yes	329	258		236	0.0805	0.0553	0.4284	0.03	0.21
BH3004	Reference control	49	145	323	No				0	0.3529	0.0165	0.0728	0.24	1.00
GH3007	Hypophysectomized	49	157	326	Yes	244	119		195	0.0253	0.0117	0.1468	0.02	0.08
GH3006	Hypophysectomized[3]	49	136	300	Yes	240	240	367	191	0.1497	0.0539	0.5005	0.06	0.25
GH3005	Control	49	125	305	Yes	240	212	367	191	0.1508	0.0432	0.4357	0.07	0.29
GH4144[2]	Reference control	85	155	352	Yes				0	0.2365	0.0530		0.15	1.00
BH3804	Hypophysectomized[4]	85	134	343	Yes	156	202	375	71	0.1624	0.0508	0.3130	0.08	0.53
W3806	Hypophysectomized	85	149	343	Yes	205	130		120	0.0417	0.0124	0.0773	0.03	0.20
GH3805	Control	85	133	333	Yes	156	183	362	71	0.1011	0.0384	0.353	0.05	0.33
						Males								
BH2981	Reference control	39	117	281					0	0.3770	0.8360	0.4790	0.32	1.00
BH2980	Hypophysectomized	39	122	287		72	108	291	33	0.1610	0.2097	0.2980	0.15	0.47
BH2978	Control	39	114	284		72	281	365	33	0.3711	2.4750	3.1007	0.14	0.44
GH2904	Reference control	40	112	284					0	0.3373	1.2683	0.6412	0.30	1.00
W2905	Hypophysectomized	40	115	273		225	157	300	185	0.0667	0.1615	0.4119	0.04	0.13
W2907	Hypophysectomized	40	121	277		225	157		185	0.0586	1.3580	1.529	0.037	0.12
W2906	Control	40	108	274		225	466	454	185	0.1465	3.2206	2.941	0.03	0.10
BH4207[2]	Reference control	41	107	284					0	0.4213	0.9875	0.5246	0.38	1.00
W2873	Hypophysectomized	41	116	291		282	112		241	0.0226	0.2494	0.3525	0.02	0.05
GH2877	Control	41	88	269		355	657		314	0.0856	3.5024		0.01	0.03

[1] At this time the hypophysectomized rats were operated upon and the rats designated as 'reference controls' were killed.

[2] Not a littermate of the animals in this group.

[3] Partially hypophysectomized. The anterior pituitary weighed 0.0041 gram; that of the control, 0.0105 gram.

[4] Partially hypophysectomized. The anterior pituitary weighed 0.0023 gram; that of the control, 0.0082 gram.

which the anterior and posterior hypophysis is completely ablated, the pituitary stalk and diaphragma being left intact (Smith, '27, '30). These hypophysectomized rats are from thirteen different litters, in each litter there being at least one control autopsied at the termination of the experiment and one which was autopsied at the time of hypophysectomy (reference control). The age of the animals at the time of operation varied from thirty-nine to ninety-three days; the period between operation and autopsy, from seven to 282 days. All the animals were autopsied while in a healthy condition, so that the condition of their thymi was not influenced by disease or by terminal cachexia.

Although in the animals operated upon at the more advanced ages (eighty-five and ninety-three days) the thymus had attained its maximum size, it is apparent from the data of Donaldson ('24) and Hammett ('25) that no absolute decrease in weight had occurred at the time of the hypophysectomy. The thymi of the rats operated upon at the earlier ages had not attained their maximum sizes.

The thymus normally involutes slowly. Because of this, the thymi of the rats which were autopsied only a short period after hypophysectomy show more decisively the effects of pituitary ablation than those of the long survivals and will be discussed first. In the two females which were allowed to live for the shorter periods (seven and twenty-seven days) it is seen that, in contrast to the control, not only did their thymi not increase further in size, but decreased. In spite of the fact that no increase in general body weight has taken place in the operated animals, nevertheless the decrease in thymus weight is sufficient to make the percentage weight of the thymus on body weight less than in the controls. This decrease in thymic weight is even more strikingly shown with the relative percentage weights. In the one male which was autopsied shortly after pituitary ablation (thirty-three days) the thymus, in contrast to that of the control, had decreased markedly in weight—a decrease, however, which is not shown by the percentage weights, due to the fact that the control had more than doubled its general body weight in this period.

The thymi of animals allowed to live for longer periods after hypophysectomy (120 to 282 days) show an actual loss in weight greater than in the control animals, weighing, with but one exception, one-half or less than one-half of the thymi of the controls. The percentage weights, however, of the thymi of the operated animals figured on body weights are nearly identical with those of the controls, due to the failure of the rats to increase in general body weight after ablation of the hypophysis. The thymi of both the operated and control rats show a marked decrease in proportion to body weights in these longer survivals, as is shown by the comparisons with the reference controls.

Two cases of partially hypophysectomized rats are entered in the table. These are of interest for two reasons. They show that the greater absolute loss in the weights of the thymi in the hypophysectomized as compared with the control animals is due actually to apituitarism, and not to some other injury inadvertently inflicted during the operation, for they were subjected to the same operative procedure employed in the cases of total removal of the anterior and posterior hypophysis. In each of the cases a small lateral fragment of the anterior hypophysis escaped removal. In spite of the fact that all of the posterior lobe and over one-half of the anterior lobe was removed in these animals, their thymi show an absolute loss in weight no greater than those of the controls. These animals are also of interest in indicating how severe a loss of anterior-pituitary tissue can be suffered without impairing general body growth or the reproductive system. In spite of the great reduction in anterior-lobe tissue, the rate of general body growth and the ovarian and uterine weights were not decreased. The number of cases is too few to justify generalization. It will be treated in a subsequent paper in which data from a considerable number of partial hypophysectomies will be presented.

The data thus show that following the total ablation of the anterior hypophysis the thymus begins to regress in weight almost immediately and that its absolute loss in

weight in long survivals is much greater than that in normal rats. In proportion to general body weight, however, its weight is nearly the same in the two types of animals, due to the fact that the controls continue to grow, whereas hypophysectomized animals not only do not gain, but usually decrease in general body weight.

The hastened involution of the thymus consequent to hypophysectomy is usually, though not invariably, shown structurally. In figure 4 maximal involution is shown. In this rat, killed 236 days after hypophysectomy, the thymus has suffered a profound invasion and replacement by adipose tissue, and the demarcation between the cortex and medulla has almost entirely disappeared. In figure 2 is shown a section of thymus of a rat autopsied 154 days after hypophysectomy in which, on the other hand, the structure of the thymus is almost identical with that of its unoperated littermate sister. Although the weight of this thymus was about one-third that of the control, nevertheless the normal cortical-medullary separation and proportion are retained. The thymi of all the other operated rats show degrees of involution intermediate between those illustrated in figures 2 and 4, except in one case. In this case the operated rat (W2873 ♂) was autopsied 241 days after hypophysectomy at the age of 282 days. Its thymus shows a profound involution, whereas that of the control, although it was not autopsied until seventy-three days later, shows structurally but slight changes.

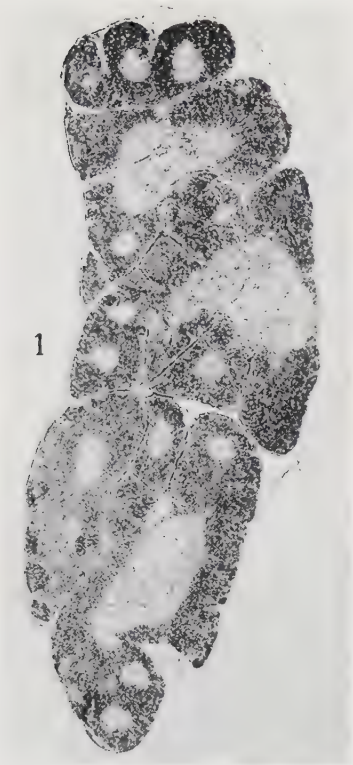
Each figure is from a cross-section through the middle third of the thymus. × 34.

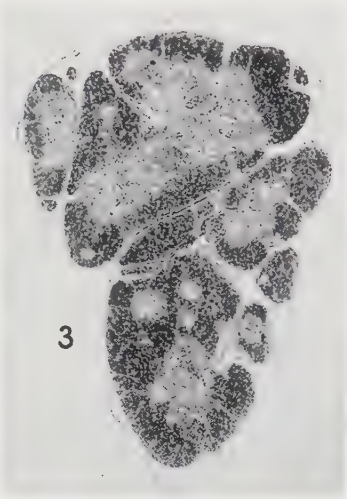
Fig. 1 From rat (BH3903♀), a littermate control of BH3901♀, the thymus of which is shown in figure 2. Age at autopsy, 200 days.

Fig. 2 From rat BH3901♀, hypophysectomized when forty-five days old and autopsied 155 days later. Although the weight of the thymus of this animal was but 37 per cent that of the control, but slight indications of involution are shown structurally.

Fig. 3 From rat BH2825♀, a littermate control of BH2827♀, the thymus of which is shown in figure 4. Age at autopsy, 325 days.

Fig. 4 From rat BH2827♀, hypophysectomized when ninety-three days old and autopsied 236 days later. Pronounced involution is shown structurally.





Figures 1 to 4

Thus, although the thymi may show pronounced structural changes of involution following hypophysectomy, these are somewhat inconstant and in most animals are not as great as would be expected from the decrease in the weight of the gland. The effects may indicate, it seems, that the thymus responses are not due to a direct effect of the pituitary ablation upon the thymus, else the structural changes should be constant as in the case of the thyroids, adrenal cortex, and gonads. The variability indicates that the changes may be due to several factors which are induced by the hypophysectomy and the resultant response dependent upon which of these is predominant. Aging of the rats obviously would play no part, since it would operate equally in control and hypophysectomized animals.

The ablation of the anterior hypophysis induces an atrophy of the gonads so profound that any secretory activity of these atrophied organs would not seem possible. Since the removal of the gonads tends to retard, though not ultimately prevent, the involution of the thymus, this gonadal atrophy after hypophysectomy must operate in retarding the involution of the thymus.[3] A second factor present as a result of hypophysectomy which experimental work indicates would influence thymic involution is a thyroid deficiency. The thyroids become atrophic subsequent to hypophysectomy. The low basal rate indicates that their functional activity is greatly reduced. That thyroid ablation hastens thymic involution in young rats seems certain from the work of Hammett.

A third factor by which the hypophysectomy probably indirectly influences the thymus is through an insufficiency of the adrenal cortex. The adrenal cortex of the hypophysectomized rat is greatly reduced in amount and is structurally abnormal. This, together with the lowered resistance of these animals and the absence of compensatory hypertrophy

[3] It does not seem necessary to refer extensively to the literature in regard to the responses of the thymus in these various conditions. An excellent statement of the literature is given by Marine, Manley, and Baumann. More recent literature is given in the review of E. Thomas in Hirsch's "Handb. d. inneren Sekretionen" and in the paper by Courrier.

of the remaining adrenal after unilateral adrenalectomy, indicates a pronounced lowering of the cortical activity. Although work upon the effect of adrenal insufficiency on the thymus is still meager, findings reported by Jaffe ('24) from an extended series of rats, together with the results of Marine and his coworkers in the rabbit, make it seem certain that we have here a factor which would tend not only to retard thymic involution, but to cause an hyperplasia.

A fourth factor appearing as a result of hypophysectomy which undoubtedly directly affects the thymus is the inanition which develops following the ablation of the pituitary. In all cases the food intake is greatly reduced and activity diminished. In the rats operated on at the ages used in this study outspoken cachexia does not develop, although some loss in weight is usually observed. The extent of the inanition is not mensurable and undoubtedly varies quite widely. It is to the variability of this factor that I am inclined to attribute to a considerable extent the variability in the structural response of the thymus following hypophysectomy. That the thymus is labile and extremely responsive to general body conditions has been demonstrated in the extensive studies of Hammar. Courrier points out in his thyroid work that because of "la grande fragilité du parenchyme thymique" it is not justifiable to consider a response of the thymus as a specific effect of a glandular ablation. Although Jackson ('15) in his series did not find that inanition produced involution of the thymus, this he believed was due to involution having already taken place, since his rats were approximately a year old at the time of his experiment. From the evidence of Hammar and others it seems certain that inanition is an important factor producing involution of the thymus, and consequently, since it is present in varying intensities in hypophysectomized rats, it will in varying degrees affect the thymi of these animals.

In the foregoing I have indicated four factors induced by hypophysectomy by which the involution of the thymus would be influenced, in addition to any direct effect which the loss

of the anterior-pituitary secretions may have. Their influence, it seems, would be sufficiently great to make unnecessary the assumption of any specific influence of the anterior hypophysis upon the thymus in order to account for the hastening in its involution after hypophysectomy.

SUMMARY

The thymi of hypophysectomized rats have been compared as regards weights and structure with those of controls and reference controls.

The thymi of rats cease to grow immediately after the ablation of the hypophysis, and regress in weight. In the long survivals the thymi weigh less than one-half those of the controls—a difference, however, which is not shown by percentage weights on body weights, due to difference in the rate of general body growth in the two types of animals. Structurally, the involution of the thymi in the hypophysectomized rats is usually, though not invariably, more advanced than in the controls.

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NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

A TEXTBOOK OF HISTOLOGY, by Alexander A. Maximow, completed and edited by William Bloom. 1930. Size, $6\frac{3}{4} \times 10$ inches. Pages xiii + 833. 604 illustrations, some of which are in color. Index. Cloth. Price, $9.00. W. B. Saunders Company, Philadelphia.

This text-book of histology was planned and partly written by Professor Maximow. After his death, in December, 1928, the rough manuscript and notes were turned over to Dr. William Bloom to be developed and prepared for publication. In completing the book Doctor Bloom has followed as far as possible the lines laid down by Professor Maximow, basing the figures and text mainly on human material and emphasizing the functional aspect of structure. He has had the aid of numerous scientists as consulting collaborators, to whom he he gives credit in the preface. The final product is an outstanding work, up to date in its treatment of histology and generously illustrated with figures and plates well chosen and executed. Each chapter is followed by a brief list of the more important sources of reference.

LEONARDO DA VINCI, THE ANATOMIST (1452–1519), by J. Playfair McMurrich. (Publication no. 411 of the Carnegie Institution of Washington.) 1930. Size, 7×10 inches. xiii + 265 pages. 89 illustrations. Index. Glossary. Bibliography. Cloth. Price, $6.00. The Williams & Wilkins Company, Baltimore.

Of all the authors who have undertaken critical analyses of Leonardo da Vinci none has been better fitted for the task than is Doctor McMurrich, in whom are blended the technical, historical, and aesthetic qualifications essential for doing justice to the subject. As one of the foremost anatomists of our times, the author is competent to evaluate the truly great contributions made to anatomy by the artist and scientist of the fifteenth century. Before and during Leonardo's time ideas of human structure were vague, and such pictures as existed were schematic and inaccurate, and the dogmatic Galenic traditions were universally accepted as incontrovertible facts by medical men and scholars. Leonardo, filled with insatiable curiosity regarding all manifestations of nature, escaped some of the current misapprehensions through his ignorance of medical teachings and his study of anatomical specimens. He observed closely and usually drew exactly what he saw, although occasionally misled into following hearsay, as, for example, when he pictured the septum of the heart showing perforations which do not exist. His drawings and notes show an accuracy surprising when compared with contemporary efforts. The illustrations in this book emphasize the contrast. It is a volume every anatomist would enjoy. It is beautifully printed and bound as befits its high quality.

STUDIES ON THE STRUCTURE AND DEVELOPMENT OF VERTEBRATES, by Edwin S. Goodrich. 1930. Size, $5\frac{3}{4} \times 8\frac{3}{4}$ inches. Pages xxx + 837, including 42 pages of bibliography. 754 illustrations. Index. Cloth. Price, $10.00. The Macmillan Company, New York.

As a book for advanced students and people engaged in teaching and research this volume deals rather fully with certain subjects and problems of special interest not fully treated in current text-books, emphasizing morphogenesis and

comparative anatomy. It sums up the facts already known, discusses their significance, and calls attention to the points where knowledge is deficient and should be helped out by further research. It is written mainly from the morphological point of view, without, however, losing sight of function. An excellent classification of vertebrates (Amphioxus to Homo) lists the subphyla, branches, classes, subgrades, subclasses, orders, groups, and divisions. The bibliography contains a selection of the more important and more recent works. The chapter titles are as follows: Vertebral Column, Ribs, and Sternum; Median Fins; Paired Limbs; Limb Girdles; Morphology of the Head Region; The Skull; Skeletal Visceral Arches and Labial Cartilages; Middle Ear and Ear Ossicles; Visceral Clefts and Gills; Vascular System and Heart; Air-bladder and Lungs; Subdivisions of the Coelom, and Diaphragm; Excretory Organs and Genital Ducts; Peripheral Nervous System and Sense Organs.

THE BUREAU OF ENTOMOLOGY, Its History, Activities and Organization, by Gustavus A. Weber. (Service Monograph of the United States Government no. 60.) 1930. Size, 6 × 9 inches. Pages xii + 177. Bibliography. Index. Buckram. Price, $1.50. The Brookings Institution, Washington.

Another of the Government's interesting monographs. This describes one of the scientific branches of the Department of Agriculture, which conducts research studies of insects in their economic relation to agriculture and forestry, agricultural products, and the home, and their bearing on the health of man and animals.

THE BUREAU OF HOME ECONOMICS, Its History, Activities and Organization, by Paul V. Betters. (Service Monograph of the United States Government no. 62.) 1930. Size, 6 × 9 inches. Pages x + 95. Index. Bibliography. Buckram. Price, $1.50. The Brookings Institution, Washington.

The Bureau of Home Economics has two functions, to study practical home problems, and in this manner aid in improving and bettering living conditions, and to study the relative utility and economy of agricultural products for food, clothing, and other uses in the home. The public should be educated to appreciate these monographs which explain the organization and operation of the Government.

LABORATORY MANUAL OF GENERAL BIOLOGY, by George G. Scott. 1930. Size, 5¼ × 8¾ inches. Pages xi + 125. Buckram. Price, $1.00. Thomas Y. Crowell Company, New York.

The laboratory period is the student's main opportunity to acquire an acquaintance with the science of biology. The author believes that the laboratory should be a pleasant place and that the work there should be as fascinating as any sport. This manual is planned to arouse and stimulate the student's interest as he investigates for himself the phenomena of living creatures. It was written primarily to supplement the text-book "Science of Biology" by the same author, and consists of questions and laboratory directions covering the course. The lessons for the first semester bring the work up to the phylum Chordata. The lessons for the second semester include studies of the Chordata, especially the Vertebrata; general biology; and the biology of man.

THE EFFECT OF TOTAL THYROIDECTOMY ON THE STRUCTURE OF THE PITUITARY GLAND IN THE RABBIT

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ONE COLORED PLATE (TWO FIGURES)

INTRODUCTION

Practically all observers are agreed that thyroidectomy affects the pituitary body and that the latter increases in size after this operation. All but Herring ('08) agree that the chief change is in the anterior lobe. While some think that it is chiefly in the chromophobe cells, others (Satwornitzkaja, '26) regard the basophiles as the cells chiefly affected, and Cimoroni ('07) considers that the eosinophile cells are markedly increased.

The reason for these disagreements is undoubtedly to be found in the fact that, notwithstanding much investigation of the most searching cytological sort, the functional composition of the glandular portions of the pituitary body is still imperfectly understood.

Since the work of Lothringer ('86), Flesch ('86), and Rogowitsch ('89) it has been known that the anterior lobe contains at least two types of cells. To these Schönemann ('92) added a third in the so-called basophile (cyanophile) cell, and the work of Tilney ('14) and Atwell ('26) set aside the pars tuberalis as different from the anterior lobe, while the observations of Maurer and Lewis ('22) and of Rasmussen ('21) recognized special characteristics in certain cells of the pars intermedia of the pig and woodchuck.

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Only one of these cells, however, has been clearly recognized as a differentiated cellular type constant in its occurrence throughout vertebrates. That is the so-called eosinophile cell of Schönemann, better termed alpha cell, as proposed by Bailey ('28). This cell is among the earliest to be differentiated from the common cell mass in the embryo, has been recognized in every pituitary body which has been examined, and is easily recognized by perfectly definite structural and tinctorial characteristics.

The basophile cell, or beta cell, however, while constituting in the human pituitary body and in that of the pig, dog, ox, and woodchuck a clearly defined cellular type, has been frequently identified by vague tinctorial characters which neither establish it as a distinct cell type in other species nor separate it definitely from certain cells belonging to the chromophobe series. In the species mentioned above this cell is characterized by the presence in its cytoplasm of discrete granules which stain with the less disperse dyes such as the aniline blue of the Mallory stain, acid violet of the Maurer and Lewis techniques, or the kresofuchsin of Kraus ('12). It is apparent from a perusal of the literature that cells have been identified as basophile cells when their protoplasm took up these stains in a diffuse way with no suggestion of special granules. Other characters often ascribed to the basophile or beta cells are possessed in some degree by all other components of the anterior lobe. This statement refers to the presence of vacuoles in the cytoplasm, to the presence of a cytocentrum (macula) associated with a vacuome or Golgi apparatus, and to mitochondrial and nuclear characters.

The special cells of the pars intermedia described by Maurer and Lewis ('22) in the pig and by Rasmussen ('21) in the woodchuck have not been generally recognized by other observers, but have, in the species just mentioned, a character which should make their recognition easy if suitable technical precautions are observed. These cells are of large size and contain a variable amount of a finely granular cell product which occupies definite compartments in the cell

separated by cytoplasmic partitions. This substance Maurer and Lewis showed to be extremely labile, dissolving out of the cell when fixation was delayed. These cells also show a quality which is not exclusive to them, but forms one of their conspicuous characteristics, namely, a large mass of cytoplasm near the nucleus, rich in mitochondria, containing the vacuome and the cytocentrum.

The cells which are commonly termed chromophobe, those cells of the pars intermedia which do not belong to the category just described, and the cells of the pars tuberalis constitute a group of cellular elements containing no one knows how many functional types, since up to the present it has not been possible to define them with sufficient accuracy so that an observer can recognize them from one another when he sees them. Rogowitsch and all subsequent observers recognized that some were large and some small, and Maurer Lewis demonstrated a special kind of chromophobe cell in the anterior end of the anterior lobe of the pig's pituitary, but, in general, while there is much prolixity of description, neither mitochondria, nor Golgi apparatus, nor secretory content has sufficiently defined any one of these cell types so that an experimenter can distinguish it with certainty from other similar cells or trace its changes under experimental conditions. Moreover, the relations of the cells constituting the chromophobe series to the chromophile cells of both types remain obscure. Whether the latter reproduce their kind, are recruited from the chromophobe group, or are transformable one into another is unknown.

The irregular distribution of the various chromophile cells in the anterior lobe is also undoubtedly a source of much confusion where the work is not controlled by the serial-section method or by careful quantitative study like that of Rasmussen on the woodchuck. It is not difficult to be misled by the comparison of sections from an experimental and a control gland which are not taken from the same part and the same plane of the gland.

In spite of these handicaps which still remain, I have endeavored to ascertain by experiment in the rabbit whether removal of the thyroid gland produces changes in the pituitary body and the cells that are involved in this change.

After a preliminary series of experiments on the adult animals which yielded positive results, it was decided to use for the experiment young males, reserving their male litter mates for controls. In the experimental animals both lobes and the isthmus of the thyroid gland were removed under ether anaesthesia in the usual way, leaving the external parathyroids in situ to avoid postoperative tetany. The experimental animals were then kept in the same cages with their male litter mates under the same conditions as regards food, etc., until a sufficient time had elapsed The animals were then killed by heart puncture, the calvarium removed, the pituitary body carefully extracted from the sella, placed in a weighing tube and carefully weighed, then fixed in Regaud's fluid. The animal was then carefully inspected to be sure that the thyroidectomy had been complete. With this end in view, the animals were perfused from the aorta with a 1-40,000 solution of Meldola's naphtol blue, which has the property of staining thyroid tissue intensely blue. All suspected tissue was then removed, fixed, embedded, and sectioned to see if it contained thyroid tissue. In one case only was this found.

The pituitary bodies after embedding were sectioned in complete series in the sagittal plane and stained by the anilin acid-fuchsin methyl-green method of Bensley usually employed for mitochondrial stain.

For the study of the normal gland and glands from adult cretins, the pituitary glands of these individuals fixed in Bensley's formalin-Zenker solution or Regaud's formalin-bichromate solution were stained in a modification of the Mallory method for connective tissue, using a 10 per cent solution of acid fuchsin in anilin water instead of the usual fuchsin solution. The iron-haematoxylin and the anilin acid-fuchsin methyl-green methods were also employed. The modi-

fied Mallory method when used on the human pituitary gland or on that of the pig or dog is particularly useful in differentiating very clearly the alpha cells, the granules of which stain red or yellow, the beta cells, the granules of which stain blue, and the chromophobe cells, which contain no specific granules, but stain in different intensities of blue corresponding to a variable content of a non-granular secretory product. Either this method or the anilin acid-fuchsin methyl-green method serves well to define the characteristics of the special cell of the pars intermedia as described by Maurer and Lewis and by Rasmussen.

THE EFFECT OF THYROIDECTOMY ON THE SIZE OF THE PITUITARY GLAND IN RABBITS

Our results obtained by comparison of weights of the pituitary gland of thyroidectomized animals with those of their litter mates of the same sex confirm the earlier quantitative results of Stieda ('90) and the impressions of numerous other investigators that thyroid deficiency results in an increased size of the pituitary body.

TABLE 1

ANIMAL AND SERIES NO.	INITIAL WEIGHT	MAXIMUM WEIGHT	FINAL WEIGHT	TIME ELAPSED AFTER OPERATION, DAYS	WEIGHT OF HYPOPHYSIS
Series 1:					
Rabbit P	410	1140	960	81	0.022
Control	Not taken	1570	1420		0.0188
Series 2:					
Rabbit S	550	1230	1230	110	0.0246
Rabbit T	550	1300	1270	79	0.0185[1]
Control	530	1340	1220		0.0195
Series 3:					
Rabbit U (female)	320	640	500	64	0.0225
Control	460	1020	1020		0.0198
Series 4:					
Rabbit X	400	820	550	90	0.022
Control	360	820	820		0.016
Control	340	920	920		0.015

[1] Rabbit T was found, on reoperation on the seventy-eighth day, to have some residual thyroid tissue. This was removed, but the animal died the following day, and the weight of the hypophysis showed that the condition of the latter was as in a normal animal.

With the exception of rabbit U, all the animals used in this study were males, and the members of each series were litter mates.

These experiments demonstrate that complete thyroidectomy results in increase in weight of the pituitary gland. It remains to be seen to what tissue element of the gland this increase is due. In order to do this, it is necessary first to consider the structure and cell composition of the normal gland.

THE NORMAL PITUITARY GLAND OF THE RABBIT

The normal pituitary gland of the rabbit consists, as in other mammals, of the following parts: pars distalis, or main glandular lobe; pars infundibularis, or epithelial portion, which surrounds the pars nervosa; pars tuberalis, extending forward on the under surface of the brain; and pars nervosa.

The pars distalis constitutes the main glandular mass usually known as anterior lobe. It is partly separated from the pars infundibularis by the residual lumen, a cavity derived from the original pouch of Rathke, but is continuous with the infundibular part at the extremities of this cavity. It is also continuous above with the pars tuberalis.

The cell types found in the anterior lobe are well illustrated by figure 1, which is drawn from a normal rabbit gland fixed in formalin-Zenker and stained with anilin acid fuchsin and Mallory's anilin blue-orange G mixture. The conspicuous cell type is the alpha cell (eosinophile), which, in this preparation, is stained strongly with the acid fuchsin. The red stain is due to the fact that the cytoplasm is closely packed with minute granules which take the stain, filling the cell so closely in many cases from the normal gland that the individual granules are difficult to discern. In the rabbit's gland, however, it is not uncommon to find many alpha cells which have their granule content reduced to a mere line of granules along the edge. Some of the alpha cells contain granules which stain with the orange component of the dye, and this is particularly the case with clusters of small alpha cells

which appear to be young elements, or elements just beginning secretion storage.

The alpha cells in the rabbit's gland are present in all parts of the anterior lobe, but are particularly numerous in the portion of this lobe which is adjacent to the residual lumen and to the pars infundibularis and on the ventral and lateral surfaces of the lobe. The anterior portion of the lobe and the adjacent central portions contain scattered alpha cells, but they are relatively infrequent. This area constitutes the so-called triangular area of Rogowitsch. The upper border of the anterior lobe is also poor in alpha cells and is directly continuous with the pars tuberalis.

Among the deeply stained cells of the anterior lobe of the rabbit's gland may be recognized a certain number of cells which prefer the blue dye and so have the general characteristics of the basophile cell of the human gland. In these cells, however, one does not find the discrete blue-stained granules of the human gland, but the staining is diffuse. The vacuome with its associated cytocentrum is conspicuous in these cells as it is in the human basophiles, but this is merely a result of the more vivid staining of the cytoplasmic background. Moreover, these cells grade through a whole series of intermediate degrees of staining into the palely staining chromophobes of the anterior lobe. It is extremely doubtful whether these are true physiological homologues of the basophile cell of man.

The chromophobe cells which make up a considerable portion of the anterior lobe are, as indicated in the introduction, palely staining cells of indefinite character in which no clearly defined secretory substance is discernible. They vary much in size, however, and some are more cyanophile than others. The chromophobe cells of the upper and anterior border of the gland are continuous with the peculiar cell cords and tubules which constitute the pars tuberalis. There is no abrupt change of cell type in passing from pars anterior to pars tuberalis, but the cells of the latter stain less intensely than the chromophobes of the anterior lobe, and the cell cords

show a greater tendency to form central lumina—a tendency, however, which is also displayed in some degree by the chromophobes of the anterior lobe; moreover, as the sequel will show, the cells of the pars tuberalis react less to thyroid deficiency.

The residual lumen is an irregular space interposed between the pars infundibularis and the anterior lobe. It contains a creamy material which resembles thyroid colloid, but is less homogeneous. The lumen is lined by relatively undifferentiated stomodaeal epithelium, many cells of which bear cilia. Among these cells on the anterior border of the lumen a few alpha cells may intrude, and on the posterior border occasionally a special pars-infundibularis cell.

The pars infundibularis is the mass of epithelial cells surrounding the pars nervosa. It consists of a thin layer of cells covering the posterior and lateral aspects of the pars nervosa and a thick blanket of cells separating the pars nervosa from the residual lumen. The latter is the epithelial portion usually referred to as the pars intermedia.

The pars infundibularis contains cells of several sorts, only one of which is, however, specific for this portion of the hypophysis. This is the large pars-intermedia cell first described in detail by Maurer and Lewis ('22) in the pig, afterward recognized and described by Rasmussen ('21) in the woodchuck. Other observers have been unsuccessful for the most part in differentiating this cell from the other components of the pars intermedia. The reason for this is that these cells contain a type of secretion granule which is extremely labile and rapidly disappears from the cell postmortem, so that unless the pituitary gland is properly and speedily fixed after death, the characteristic content of these cells disappears. These cells, which form the main mass of the pars intermedia in the rabbit, are practically identical with those of the pig, and the description of them by Maurer and Lewis applies with full force to the rabbit's gland. The cells are large in size, and contain a variable amount of secretion in the form of very minute granules which stain

blue in the anilin acid-fuchsin Mallory method. The secretion is contained in rather large spaces in the cytoplasm, separated from one another by strands of cytoplasm which radiate from the nucleus and from a condensed mass of cytoplasm about the same size as the nucleus and alongside of the latter. This condensed mass of cytoplasm contains very numerous small rod-like mitochondria, and incloses the vacuome and the cytocentrum. The cytoplasmic condensation is so characteristic that under low powers these cells appear to contain two nuclei.

Cells of this sort are found only in the pars intermedia. The latter, however, is continuous with pars distalis at the ends of and around the residual lumen, and at these points the pars-intermedia cells may easily be mistaken for accumulations of basophile anterior-lobe cells.

The other cells of the pars intermedia are the following: 1) Indifferent stomodaeal epithelium and ciliated cells forming the posterior boundary of the residual lumen. These cells also tend to extend through the pars intermedia and separate the special cells of that part into groups. 2) Cells of low order of differentiation, of small size and palely staining, scattered among the special cells of the pars intermedia. Cells of this sort also form vesicles containing a clear colloid optically similar to that of the thyroid gland. The cells surrounding these vesicles sometimes contain small intracellular drops of similar colloid, but are most frequently free from it. Part of the wall of the colloid-containing vesicles may also be formed by the special pars-intermedia cells.

The pars nervosa calls for no special description except to remark that it contains the usual masses called hyaline bodies. These are not, however, actually hyaline, but have a finely granular structure and they stain differentially from the colloid of the pars intermedia in Mallory's connective-tissue stain, which colors the hyaline bodies red and the colloid blue, and in Mallory's phosphotungstic acid-haematoxylin, which stains the colloid pink and the hyaline bodies blue.

CHANGES IN THE THYROIDECTOMIZED ANIMALS

In the thyroidectomized animals the cellular composition of the pars distalis (anterior lobe) is profoundly changed. In the other parts of the pituitary gland—namely, pars tuberalis, pars intermedia, and neural lobe—I can detect no change. The content of colloid cysts and of hyaline bodies in the pars intermedia and pars nervosa, respectively, is within the normal range of variation, and I can therefore not confirm Herring ('08) in this respect.

The pars anterior, as indicated above, is profoundly modified. The most striking change on first study is the great reduction in the number of eosinophile cells. In the long-standing cases this amounts almost to a disappearance of eosinophiles from the anterior lobe. The triangular area of Rogowitsch becomes practically free from alpha cells, and in the posterior portion where they are normally abundant they are sparse. This is a progressive change which becomes more pronounced with the longer duration of athyroidism. The eosinophiles which remain are no longer the deeply staining granule-packed cells of the normal gland, but the granules are fewer in number and stain less intensely.

The question whether the eosinophiles are diminished by loss of specific granules or by degeneration and cell breakdown is not easy to determine, and I cannot give a final answer to it. This much is certain, that many eosinophiles show evidences of degeneration in reduction in size and in granule content and by a shrunken pycnotic nucleus, while others show vacuolation, reduction of granule content, and chromatolysis. Both are undoubtedly degenerating cells and some of the deficit is to be accounted for by this process. If there are in addition dedifferentiated eosinophiles, they are not to be distinguished from the profoundly modified chromophobe cells of the anterior lobe.

The chromophobe cells of the anterior lobe are mainly responsible for the increase in size and weight of the organ after thyroidectomy. In the thyroidectomy gland basophile cells are no longer recognizable, so, if the vaguely defined

basophiles of the normal gland were really specific cell types, they have lost their differential characters or have disappeared. All of the chromophobes, on the other hand, are markedly hypertrophied, and this cell hypertrophy is responsible, notwithstanding the fact that there is evidence of much cell destruction, for the increase in size and weight of the gland. Mitoses are quite exceptional.

The hypertrophy of the chromophobe cells is well shown in figure 2, which also shows the extraordinary diminution of alpha cells. Whether this increase in size is due to an accumulation of non-granular secretion in these cells or to increase of cytoplasm I am unable to say.

Many evidences of degeneration are found in the hypertrophied chromophobes of the anterior lobe. These vary from a moderate vacuolation of the cytoplasm to a complete breakdown of the cell. The latter is responsible for the presence in the thyroidectomy gland of large intercellular spaces which have a punched-out appearance. These spaces contain a variable amount of cellular débris.

The specific reaction of the chromophobe cells to thyroid deficiency is another evidence that these cells represent one or more types of specific secretory elements in the hypophysis, and are not merely reserve cells.

I do not find groups of special stroma cells in the rabbit like those described by Satwornitzkaja ('26) in the dog after partial thyroidectomy.

CONCLUSIONS

Complete removal of the thyroid gland in the rabbit results in an enlargement of the pituitary body. This increase in size is exclusively in the anterior lobe, and is due to a progressive hypertrophy of the chromophobe cells. There is also a progressive reduction in number of the eosinophile cells. Also, many of the hypertrophied chromophobe cells degenerate, first becoming vacuolated and then completely breaking down, leaving large gaps containing cellular débris.

There is no justification for interpreting these results as indicating an increased secretory activity of the pituitary gland in cases of thyroid deficiency—quite the contrary. The disappearance or reduction of the alpha cells indicates rather a depression of the specific secretion of these cells, and the tendency of the enlarged chromophobes to degenerate is an indication of the tendency of functionally depressed cells to complete their cytomorphosis and disappear rather than to dedifferentiate and reproduce.

The profound modification of both alpha cells and chromophobes under these conditions suggests that some of the results of thyroid deficiency may be produced indirectly through depression of the pituitary gland.

The author wishes to express his appreciation to Dr. Margaret M. Kunde for the opportunity of examining the pituitary glands of long-term cretin rabbits, and to Prof. R. R. Bensley for aid and guidance in the investigation.

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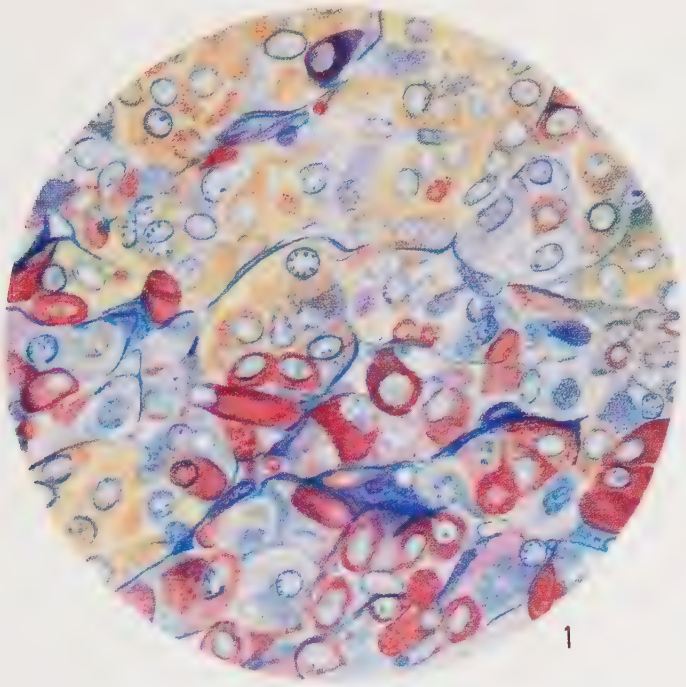
PLATE 1

EXPLANATION OF FIGURES

In the process of reproduction the granular detail of the alpha cells has been much reduced.

1 Portion of anterior lobe of the pituitary of normal adult rabbit, fixed in formalin-Zenker and stained with anilin acid fuchsin-Mallory. × 840. Shows alpha cells with red granules, other alpha cells with yellow granules, chromophobe cells, and cyanophile (basophile) cells.

2 Portion of anterior lobe of a long-term cretin rabbit. Same method of fixing and staining as figure 1. Shows reduction of number of alpha (eosinophile) cells, hypertrophy of chromophobe cells, and vacuolization and degeneration of chromophobes, leaving cavities with cellular débris. × 840.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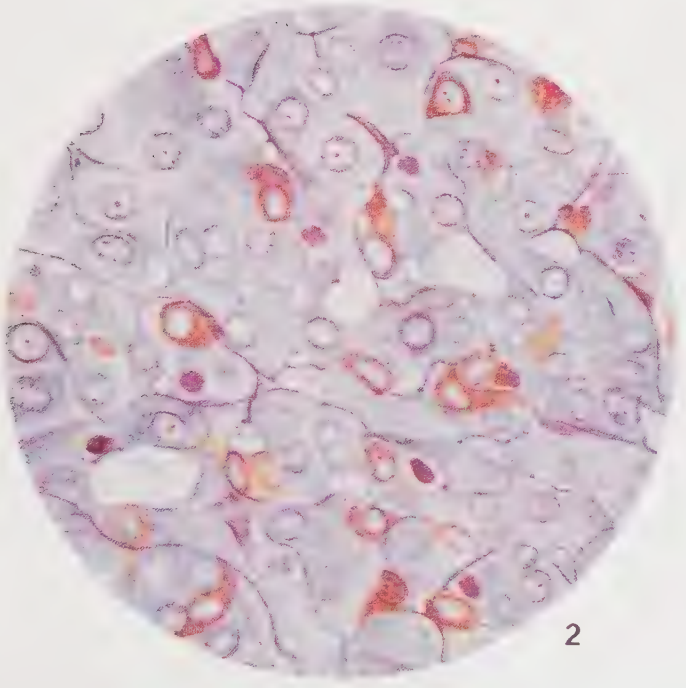
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THE STRUCTURE OF THE RENAL CORPUSCLE

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FIVE HELIOTYPE PLATES (FIVE FIGURES)

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INTRODUCTION

The recent papers of von Möllendorff ('28), Bargmann ('29), Zimmermann ('29), and McGregor ('29) indicate a renewed interest in the problem of the structure of the glomerular membrane and raise new questions as to its significance in urine formation.

Von Möllendorff's views constitute, if accepted, a real revolution in our conceptions of the structure of this membrane. Von Möllendorff succeeded, by staining with the azan modification of Mallory's connective-tissue stain, in demonstrating on the surface of the glomerular capillaries a protoplasmic network radiating from the cell bodies of the glomerular epithelium in such a way as to form a basket-like structure only partly covering the free surfaces of the capillaries. Accordingly, he compared these branched cells, which he conceived to form a syncytium, with the pericytes described by

Zimmermann and others on the surface of ordinary capillaries, and regarded the capillary of the glomerulus as essentially similar to those found elsewhere in the body.

This conception, which has been accepted also by Bargmann, who worked with similar methods, rejects the previous deductions based on embryological study, according to which the capillaries are covered by a continuous layer of epithelial cells derived from the wall of the renal vesicle. It also rejects the glomerular epithelium as a significant factor in determining glomerular permeability.

Von Möllendorff, by means of the azan method, distinguished three layers in the glomerular capillary: the covering cells, forming a network over the surface of the capillary; a basement membrane; and an endothelium. He said that approximately 90 per cent of the nuclei visible in a section of a glomerulus were epithelial nuclei, the other 10 per cent only being endothelial nuclei.

Zimmermann ('29), although the first to demonstrate in the glomerulus branched cells resembling pericytes and the author of much of the important literature on this topic, does not accept von Möllendorff's conception, but describes in the human kidney a continuous investment of epithelial cells, although he cannot decide whether this consists of separate cells or of a nucleated syncytium.

McGregor ('29), whose conclusions in regard to these problems are strengthened by her experience in studying pathologically changed glomeruli from which much valuable information may be obtained, considers that the glomerular epithelium is a continuous layer of separate cells, that it rests on a basement membrane continuous with that of the capsule of Bowman, and that the glomerular capillaries are lined by a continuous endothelium.

Zimmermann and McGregor also devote some attention to the interesting question of the structure of the capsule of Bowman. They concur in considering this capsule to consist of the following three layers: capsular epithelium, a transparent basement membrane, and a layer of reticular connective-tissue fibers.

Volterra ('28), who studied the structure of the glomeruli by means of the silver methods of Achucarro and del Rio Hortega, considers the so-called basement membrane of the capillaries of the glomerulus to be composed of a fine network of argyrophile fibers (reticulum) embedded in a homogeneous ground-substance. The figures which he publishes of this condition are, however, not convincing, in view of the work of Allen ('27), Krauspe ('22), and Luna ('21). He suggests that the network noted by von Möllendorff may be really due to connective-tissue fibers, and leaves the question of the nature of the glomerular epithelium unsettled.

Zimmermann ('29) also describes the connective tissue of the human glomerulus, but in different terms from the description of Volterra. According to Zimmermann, the connective tissue is in the form of a narrow core branching into the lobes of the glomerulus from the vascular pole and containing among its fibers numerous fibroblasts.

Vimtrup ('26), who studied the human glomerulus in the embryo and in the adult by means of special staining methods, also considers that the glomerular epithelium is a continuous covering for the capillary lobes and that it extends down between the individual lobes of which the glomerulus is composed, instead of constituting a continuous covering for the glomerulus as a whole, as some have believed.

Doubts as to the nature of the glomerular epithelium and capillary endothelium in the glomerulus have arisen chiefly because of the unsatisfactory results obtained in attempts to delineate the cell outlines by means of silver-nitrate impregnation or by staining with iron haematoxylin. So far, Nussbaum appears to have been the only investigator who has succeeded in getting impregnations of both structures by means of silver nitrate. He finds that special precautions are necessary in the frog to insure successful results, but when these are observed the capillaries are seen to be lined by a continuous layer of endothelial cells outlined in black and to be covered with a similar continuous sheet of cells.

The results obtained by other investigators with the silver-nitrate methods have been almost universally negative. Hence the tendency to regard the endothelium and epithelium of the glomerulus as a syncytium and to question its completeness.

We have found that the method of silver impregnation introduced by one of us (R. D. B., '29) for the study of the structure of the efferent vessels affords unusually good conditions for the study of the glomerular epithelium. The acid silver-citrate solution with which the kidney is perfused swells the protoplasm of the glomerular epithelial cells and makes thin sheets of it apparent over the surface of the glomerular capillaries where, under ordinary conditions of fixation, it would be difficult to distinguish it from the basement membrane of the capillary. Moreover, while complete impregnations indicating the outline of the epithelial cells of the glomerulus are rare, partial successes are frequent, and even where the amount of impregnation is slight, the cells are still separated from one another by rows of dots of precipitated silver which serve equally well to determine their boundaries. Moreover, the capillary loops in material so fixed by perfusion with acid silver citrate are fully expanded and do not have that shrunken aspect which so often hinders the study of glomeruli fixed by immersion.

We have also obtained instructive preparations from kidneys perfused, after first washing out the blood with sodium-citrate solution, with the photographic sensitizing solution used in the kallitype process. This solution gives excellent and instant fixation of the glomeruli, and after appropriate development shows an excellent silver impregnation of the endothelial membrane of the capillaries. Often this impregnation of the endothelial membrane is the exclusive silver deposit in the glomeruli, and in thick sections of such glomeruli it is possible to follow with the binocular microscope the branches of the glomerular capillaries throughout the glomerulus. The outlines of the endothelial cells of the glomerulus are, however, not visible after this method.

In his study of the efferent vessels of the glomeruli, Bensley ('29) found that the outlines of the endothelial cells of the afferent vessels and efferent vessels were clearly visible in black lines of silver, but that after their entry into the glomerulus the clear outlines usually extended only to the beginning of the primary branches, and in the capillaries themselves there was usually only diffuse staining. In a few instances, however, there was an indication of endothelial outlines in the capillaries themselves, particularly in the immediate branches of the afferent and efferent arteries. Numerous attempts to secure more complete impregnation of these cells outlines yielded no better results until the opportunity came to apply the method to the human kidney, which we were enabled to do through the courtesy of Dr. Charles B. Huggins, who placed at our disposal a human kidney removed at operation. In this kidney, perfused with citrate solution and acid citrate of silver, a few normal glomeruli revealed a partial impregnation which was comparable to that obtained by Nussbaum in the frog.

In the study of the material much aid was afforded by the application of the ammonium silver-oxide method for the impregnation of connective-tissue fibers followed by the azan modification of Mallory's connective-tissue stain—a combination introduced by Maximow for the study of fibers originating in tissue culture. This combination served to distinguish between the fibers of connective tissue when present and the basement membranes of the capillaries, which stain similarly in the Mallory stain. It also enabled us to distinguish the structureless membrane of the uriniferous tubule and of the capsule of Bowman from the overlying reticulum.

The latter purpose was, however, better served by following the impregnation with ammoniacal silver oxide by counterstaining in acridin red. After this process, the reticulum fibers were stained black and the basement membranes a clear crimson.

For the study of the glomerular epithelium it was necessary to cut sections of silver-impregnated kidneys hardened

in formalin and embedded by the usual methods in paraffin. These sections, in order to avoid confusion, were cut 2 μ in thickness, and counterstained after photographic development either by the azan process or with haematoxylin followed by acridin red or pyronin. These methods gave an intense staining of the protoplasm of the glomerular epithelial cells, which were often in addition clearly outlined by narrow lines —not continuous, but dotted—of silver deposit.

The study of these preparations shows that the glomerular membrane, in accordance with the views of McGregor, consists of three distinct layers: an epithelium, an optically structureless basement membrane, and an endothelium. The glomerulus also contains a variable amount of reticulum (argyrophile fibers).

METHODS

A. The silver-citrate method

1. The kidney is perfused from the aorta, in the case of small mammals, or from a branch of the renal artery, in the case of the human kidney, with a 1 per cent solution of sodium citrate.

2. The kidney is perfused with Bensley's acid silver-citrate solution (Bensley, '29).

3. The kidney is sliced into 10 per cent formalin, where it remains for twenty-four hours to complete fixation.

4. The pieces are run through graded alcohol, embedded in paraffin, cut, and mounted on slides in the usual way.

5. The sections are run through benzol, absolute alcohol, etc., to water and developed in any photographic developer in full daylight.

6. Wash thoroughly in water and counterstain by the azan modification of Mallory's connective-tissue stain or in acridin red.

7. Wash thoroughly, dehydrate, clear, and mount in balsam.

B. The kallitype method

1. Proceed as in the silver-citrate method, except that the kidney is perfused with the following solution, instead of the silver-citrate solution:

Ferric oxalate, 20 per cent solution,	30 cc.
Ferric potassium oxalate, 6⅔ per cent,	15 cc.
Ammonia-oxalic solution (oxalic acid, 13 grams; ammonia, 5 cc.; water, 100 cc.),	2 cc.
Potassium-bichromate solution, 1: 16,	4 drops
Silver nitrate,	2.4 grams

2. After sectioning in paraffin, the sections are run down to water, exposed to light, and developed in the following solution:

Borax,	10 grams
Sodium potassium tartrate,	7.5 grams
Water,	100 cc.
1 per cent potassium-bichromate solution,	10 cc.

3. Wash in water, fix in thiosulphate solution, and counterstain according to the azan method, or stain in hæmatoxylin followed by acridin red. Wash, dehydrate, clear, and mount in balsam.

C. The silver-oxide method

For this method, materials fixed in Zenker, formalin-Zenker, alcohol, or formalin may be employed, and the sections may be cut after embedding either in celloidin or paraffin. If paraffin sections are employed, great care must be taken in cleaning the glassware, since sections tend to float off the slide when being passed through the solutions. Celloidin sections handled without fastening to slides and without removal of the celloidin give excellent results. They also stain well when fastened to the slides by the clove-oil method of Maximow and the celloidin removed by alcohol-ether.

1. Sections are run down to water.

2. One per cent solution potassium permanganate, one to five minutes.

3. Five per cent oxalic-acid solution until bleached.

4. Wash and transfer to Lugol's solution.

5. Sodium-thiosulphate solution, any strength, until free iodine is removed.

6. Wash in water and place in a saturated solution of tannic acid in 95 per cent alcohol for five minutes at 56°C.

7. Rinse in distilled water to which a few drops of ammonia have been added.

8. Transfer to the ammoniacal silver-oxide solution made as follows:

1 per cent silver-nitrate solution,	20 cc.
40 per cent sodium-hydroxide solution,	4 drops
Ammonia drop by drop until precipitate redissolves. Dilute 1 to 10 with distilled water for use and warm to 56°C.	

Leave sections in this solution five to ten minutes.

9. Wash in water and develop in 20 per cent formalin.

10. Wash in water and tone in a 1:500 solution of gold chloride.

11. Wash and fix in thiosulphate.

12. Wash and counterstain by the azan method or acridin red.

13. Wash, dehydrate, clear, and mount in balsam.

In this method the silver-carbonate solution of Hortega may be substituted for the silver-oxide solution recommended above, if desired. The results are practically the same.

THE GLOMERULAR EPITHELIUM

As indicated in the introduction, various views have been expressed as to the nature of the glomerular epithelium; according to some, it constitutes a continuous layer of cells covering the glomerulus as a whole; others regard it as a syncytium covering the free surface of the glomerulus and the sides of the individual lobes; and recently it has been suggested that it consists of branched cells similar to pericytes which envelop the capillary loops with their processes without forming a complete covering for them.

In the glomeruli from the kidneys of Rana, Cryptobranchus, Cavia, Lepus, and Homo, we have found by the methods indicated a continuous covering of epithelial cells over all surfaces of the glomerulus, that is, over the free surfaces of the capillary loops, and on the surfaces of the lobes.

For the study of this epithelium, paratangential sections near the pole of the glomerulus are most favorable. Such a section is illustrated in figure 1, which is from the kidney of the guinea-pig. In this section it may be seen that some of the glomerular epithelium is cut parallel to the surface of the underlying capillary and that it consists of a single layer of well-individualized cells, forming a complete layer without defects, as in an ordinary epithelium. At other places the basement membrane of the capillary is overlaid for a considerable distance by a layer of cytoplasm, in some places so exquisitely thin that in the absence of adequate differential staining it would be impossible to distinguish it from the underlying basement membrane. These are obviously cells similar to those first described, but much more expanded. Inspection of these thin sheets of cytoplasm reveals thickenings at irregular intervals, which are also apparent in the figures published by von Möllendorff and Bargmann and interpreted by them as branches of the covering cells, the cell body of which they conceive to be merely that mass of cytoplasm around the nucleus.

In sections of glomerular capillaries viewed from the face so that one looks through the thin layer of protoplasm of the glomerular epithelium and the basement membrane of the vessel, the capillary is seen to be crossed by thicker and thinner protoplasmic bands, the former distinctly radiating from the larger cytoplasmic mass surrounding the nucleus. These are the bands observed by von Möllendorff and Bargmann and misinterpreted by them as evidences that the covering cell was a branched cell, the processes of which made an incomplete covering for the capillaries. As a matter of fact, they are merely thickenings in a general sheet of cytoplasm which forms a complete covering for the capillary wall.

The form of the epithelial cell covering the glomerulus varies within wide limits. On the free surfaces of the lobes the cells tend to be widely expanded, to cover rather large areas of capillary surface. Even in this situation, however, one may find small groups of cubical cells, the outlines clearly distinguishable after the silver fixation. At the vascular pole of the glomerulus groups of cubical cells are more common, and they are the rule in the sides of the lobule at points where a group of epithelial cells fills up the concavity formed by the contiguity of two capillary vessels, that is to say, in the concavity of the capillary loops. The cells in this situation are not strictly cubical cells, but may vary from this shape to cells which are many times as long in one axis as in the others. The cytoplasm of these aggregates has a flocculated appearance, as if there were two densities of protoplasm in the cell. Epithelial cells of this sort probably form in part the material which Zimmerman described as 'zwischengewebe.' The mass of cells has, as a whole, an appearance of fibrillation, due to the two densities of protoplasm in these contracted epithelial cells. They do not, however, stain blue in Mallory's connective-tissue stain and they remain negative to the silver method for argyrophile fibers. It is probably the greater resistance of the denser cytoplasm to extension that gives rise to the ridges and bands mistaken by von Möllendorff and Bargmann for cell processes.

The clusters of epithelial cells found in the concavities of the capillary loops appear in sections as clusters of nuclei with a small amount of cytoplasm, and the ordinary histological methods are not successful as a rule in defining the limits of the individual cells. In these clusters of cells the nuclei often appear to overlap, owing to the extreme irregularity of the surface on which the cells rest.

Figure 2 is a drawing of a section through the middle of a glomerulus taken at an acute angle across the afferent-tubule axis. In this drawing, which is at a lower magnification than the preceding figure, the general covering of epithelial cell protoplasm over the capillary loops and the groups of cells described in the last paragraph are well displayed.

In this figure it will be noted that the number of endothelial cell nuclei visible in a single 2 μ section of the glomerulus is extremely small and that nearly all the nuclei visible in the field are epithelial cell nuclei. Von Möllendorff thus correctly insists on the overwhelming proportion of the nuclei visible in sections of the glomerulus being epithelial nuclei.

Additional evidence that the glomerular epithelium is composed of separate cells and does not form a syncytium, and that these cells are of the ordinary cubical to squamous type touching their neighbors at all surfaces and not forming a network, is afforded by the study of occasional glomeruli in the guinea-pig in which the epithelial cells are filled with hyaline droplets. It is not an uncommon occurrence in the guinea-pig to find single epithelial cells of the glomerular epithelium crowded with large oval or spherical droplets which stain brilliant red in azocarmine. Such cells have their outlines clearly marked. In glomerulonephritis in the human subject, also, the glomerular epithelium may show every stage of this change from individual cells to groups of cells clearly defined from one another by their content of hyaline droplets and each cell touching its neighbors on all surfaces.

To sum up the foregoing descriptions: the glomerular epithelium is a continuous covering for the capillary loops of the glomerulus; it is composed of separate epithelial cells and is not a syncytium; the structures mistaken by von Möllendorff and Bargmann for cell processes are thickenings in the layer of epithelial protoplasm covering the capillaries. It may be added that mitoses may occasionally be found in the epithelium of the normal glomerulus.

ENDOTHELIUM

As indicated in the introduction, efforts to demonstrate an endothelial lining in the capillaries of the glomerular loops have mostly resulted in failure. Hence the views often expressed that the endothelium of these capillaries is discontinuous, or that it constitutes a syncytium. The occurrence of individual cells in the capillaries as a result of prolifera-

tion of the endothelium in glomerulonephritis should at least suggest caution in interpretation of the normal endothelium.

The excellent silver-nitrate method has as a rule yielded only negative results. Nussbaum ('86), however, secured by this method excellent impregnations, both of the glomerular epithelium and of the endothelium of the capillaries in the frog's kidney, by taking special precautions. Instead of washing the blood out of the vessels with a solution of sodium nitrate, as is commonly done, Nussbaum introduced normal salt solution into the anterior portion of the inferior abdominal vein and allowed it to be circulated by the frog's heart until it flowed clear of blood from the lower segment of the same vein. Then he tied the inferior vena cava, and injected 0.25 to 0.5 per cent solution of silver nitrate into the aorta until the glomeruli visible from the surface of the kidney became whitened. Then he washed out with distilled water and absolute alcohol. His figure of the glomerular loop of the frog (loc.cit., fig. 14) shows a continuous epithelium and a continuous endothelium both perfectly outlined by thin black lines. The significant feature in his successful method is that he did not exhaust the chlorides of the tissue by perfusion with another salt before injecting the silver solution.

The results of other investigators have been so uniformly negative that little weight has been laid on this successful experiment of Nussbaum.

In our experiments with silver-citrate solutions, it was noted that the intraglomerular portions of both afferent and efferent vessels were usually well impregnated and possessed an endothelium similar to the rest of the vessel, each endothelial cell separated from the next by a thick line of cement blackened with silver. This condition extended into the beginning of the primary branches of this vessel, but the capillary loops themselves were, as in the case of previous experimenters, usually negative. In some instances, however, even in the capillary loops, there was an indication of the separation of the endothelial cells by much thinner lines of cement much less definitely blackened. Such an instance has been illustrated by one of us (R. D. B., '29, fig. 1).

These results were so promising that we made a large number of perfusions in the guinea-pig and rabbit without, however, improving the quality of the delineation of endothelial cell outlines, and the results remained unsatisfactory and indefinite until we had the opportunity to apply the method to a fresh human kidney. This opportunity, for which we are indebted to Dr. C. B. Huggins, not only rewarded us with some excellent impregnations of glomerular endothelium, but also confirmed the descriptions of R. D. B. of the muscular coat of the efferent arteries. In no case did we get even in this kidney a complete impregnation of a vascular loop, but partial impregnations were frequent, and in figure 3 is shown one of the most successful. In this case the end of the afferent vessel inside the glomerulus is shown with the usual broad silver lines delineating the endothelial cells. These outlines are traceable into two small branches on the left side of the figure, extend throughout a large main branch, and are continuous to the small terminal capillary loop at the opposite pole of the glomerulus. Partial impregnations of side branches are also visible.

There is little doubt that in this case the usual type of endothelial lining is present in the whole of the capillary loop. Since, however, the kidney in which these successful results were obtained was pathological, some reservation must be made for the possibility that the endothelium seen in this instance is a regeneration endothelium. In view, however, of the frequent suggestive results obtained by the silver-citrate method in the normal kidneys of the guinea-pig and rabbit, this regeneration idea has little to commend it, and it is our opinion that the capillary of the glomerulus is lined by a continuous layer of endothelial cells which do not form a syncytium, but are separate cells.

By the use of the kallitype method, we have obtained additional evidence of the continuity of the endothelial lining, although this method is not so successful in impregnating intercellular lines. In kidneys perfused with this solution a delicate deposit of silver is made on the endothelium. In thick

sections this is such a perfect staining of the endothelial tube that the branches of the glomerulus can be perfectly followed with a binocular microscope. In very thin sections in which the silver image is first developed and the section then counterstained with haematoxylin and acridin red, it is seen that the silver deposit is a surface deposit on the endothelial cytoplasm, and accordingly in thin sections of a capillary loop this silver line is offset a minute but definite distance from the basement membrane, and is continuous for the whole length of the capillary loop.

BASEMENT MEMBRANE OF THE CAPILLARIES

As von Möllendorff and McGregor have pointed out, there is in addition to the epithelial and endothelial components of the glomerular membrane a third element interposed between these two in the form of a basement membrane which stains blue in Mallory's connective-tissue stain or in the azan modification. According to Volterra, this membrane is composed of a lamellar reticular connective tissue and shows when impregnated by the silver-carbonate method of del Rio Hortega a network of fine argyrophile fibers. McGregor, on the other hand, considers it to be the continuation over the capillaries of the similar basement membrane which is found underneath the epithelium of the capsule of Bowman and which, according to Mall and Disse, is continuous with a similar membrane surrounding the uriniferous tubule. Accordingly, it has been necessary to consider this basement membrane in connection with the general question of the connective tissue of the glomerulus and to approach the study of it by the silver-impregnation methods. The Mallory and azan methods do not discriminate in color between the basement membranes and the casual content of connective-tissue fibers in the glomerulus. Herxheimer ('13), by means of van Gieson's stain, was, however, able to stain the connective-tissue fibers red and the basement membranes yellow, and McGregor and Zimmermann have succeeded by other staining methods. The superiority of the silver method for the delineation of con-

nective-tissue fibers, particularly when it is combined with counterstaining according to the azan method as recommended by Maximow ('29), is so great that we have used this combination in preference to the other staining methods. We have also obtained excellent and instructive results by counterstaining in acridin-red sections previously treated according to the silver-oxide method. In these preparations the reticular fibers are stained black and the basement membranes red. We have used for this study the kidneys of rabbit, guinea-pig, and man. Our results are contrary to those of Volterra and in general accord with the previous descriptions of Luna ('21), Krauspe ('22), and Allen ('27). In the normal glomerulus of the species mentioned, the basement membranes of the glomerular capillaries remain negative to the silver method, and the glomeruli contain a very meager content of argyrophile reticular fibers. In the guinea-pig and rabbit delicate argyrophile fibers in small numbers enter the glomerulus at the vascular pole, where they are continuous with the adventitia of the afferent and efferent vessels, and extend as very delicate and sparse fibers into the lobes of the glomerulus.

In the normal glomerulus of man the arrangement of the argyrophile reticular fibers is much the same as in the animals mentioned, except that the number of them is greater and the fibers are coarser in the human glomerulus—except in glomeruli from newborn children, in which the details are much the same.

Figure 4 is a drawing of an uncounterstained section of a glomerulus from a man aged thirty years. The section engages the entrance of the afferent artery, but is a little to one side of it. It shows the investment of reticular fibers around the continuation of this artery inside of the glomerulus and the radiating strands of reticular tissue extending into the cores of the lobes.

Figure 5 is a glomerulus with afferent vessel from the same kidney. It shows the continuation of the adventitia of the artery into the glomerulus and the strands of reticular fibers

extending into the lobes. Both figures 4 and 5 show well the structure of the capsule of Bowman, consisting of an outer reticular layer excellently described by Luna ('21); the middle basement membrane, which, like the basement membrane of the capillaries, remains negative to the silver impregnation; and the inner layer of capsular epithelial cells.

Even in the kidney of the newborn, as has frequently been pointed out by pathologists, and in all kidneys from older human individuals, there are to be found completely fibrosed glomeruli, and one may search with confidence for the stages in the production of this structure from the normal glomeruli.

In the kidneys of two men of the age of fifty-four years, all of the glomeruli showed an increase of the content of connective tissue above the minimum already described and illustrated in the two figures. When this increase is present, the connective tissue impregnates well by the silver method and presents an appearance very similar to the figures recently published by Zimmermann ('29). His text figure 7 is an excellent example of such connective-tissue increase. In these cases, although the fibrous material impregnates with silver, it often lacks the discrete fibrillation of the normal glomerulus. In other words, it shows a tendency to hyalinization. In other cases the fibrillation may be quite distinct at all stages up to the complete fibrosis of the glomerular tuft.

In these cases of connective-tissue increase, cells which are presumably fibroblasts may be distinguished within the fibrous cords. Whether they derive from the glomerular epithelium or intrude from the adventitia of the afferent we are unable to say.

CAPSULE OF BOWMAN

Little needs to be added to the description of the capsule of Bowman which has been made in the last chapter, except to point out that it is not always possible to demonstrate by the methods which we have employed the structureless basement membrane shown in the illustrations. In two human kidneys, one from a boy of eleven years, the other from a man

of thirty years, every capsule of Bowman contained such a structureless membrane of considerable thickness as shown in figures 4 and 5. In the newborn, however, we were only able to demonstrate it in the case of cystic glomeruli, where it had been described previously by Herxheimer ('13). In two cases, each aged fifty-four years, we were likewise unable to demonstrate the membrane, though the reticular layer of the capsule was somewhat thicker in these instances than in the glomeruli from younger individuals where the membrane was prominent.

In those cases where a structureless membrane formed a prominent element of the capsular wall, it was also possible to demonstrate its continuity with a similar but much thinner membrane interposed between the bases of the epithelial cells of the uriniferous tubule and their reticular fiber investing membrane.

Much further work is necessary in the study of these structureless membranes in the human kidney, the consideration of which has been much diminished as the techniques for the demonstration of fibrillar basement membranes improved.

CONCLUSIONS

These observations sustain completely with new evidence the deductions recently made by McGregor as to the structure of the glomerular membrane and the capsule of Bowman. They show:

1. That the glomerular epithelium is a continuous layer over the whole extent of the surface of the glomerulus and its lobes.

2. That the glomerular epithelium is composed of separate cells and does not constitute a syncytium.

3. That the glomerular epithelium rests upon an optically structureless basement membrane which forms the supporting wall of the glomerular capillary.

4. That the glomerular endothelium may be successfully impregnated by silver, in which case it is seen to be composed of separate cells as in other blood vessels. The endothelium is continuous without defects.

5. That the amount of connective tissue demonstrable in the normal glomerulus of man and mammals is small, consisting of a layer of reticular fibers surrounding the vessels at their entrance, with sparse and delicate reticular (argyrophile) fibers extending into the lobes.

6. In some human kidneys the capsule of Bowman consists of three layers: capsular epithelium, structureless basement membrane, and an outer layer of reticulum. In others the structureless basement membrane was not demonstrable in the capsule.

7. A similar but thinner structureless membrane is interposed between the epithelium of the tubules and the reticular framework.

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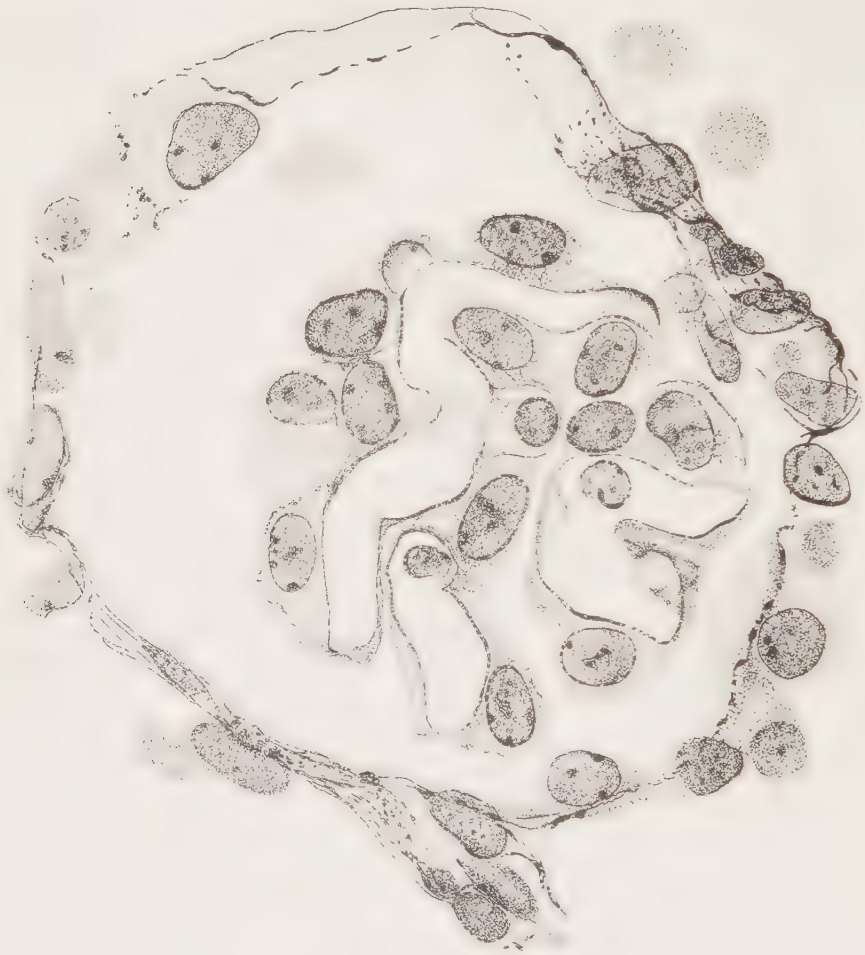


PLATE 1

EXPLANATION OF FIGURE

1 Paratangential section of glomerulus and capsule of guinea-pig. × 1425. Stained with silver citrate, developed in metol, and counterstained with haematoxylin and acridin red. Shows the glomerular epithelium made up of separate cells as in an ordinary epithelium.

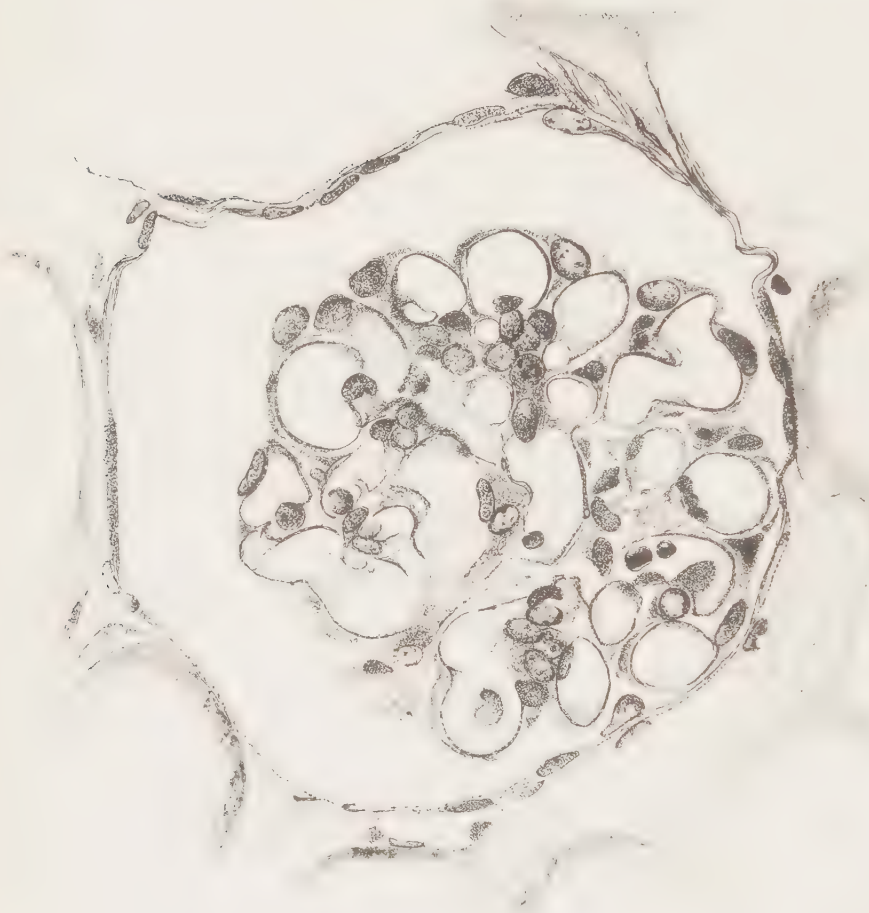


PLATE 2

EXPLANATION OF FIGURE

2 Section across guinea-pig glomerulus. × 840. Shows the clusters of epithelial cells which fill the concavities of the capillary loops, and the continuous covering of cytoplasm over the free surfaces.

PLATE 3

EXPLANATION OF FIGURE

3 Section of human glomerulus, with endothelial cell outlines stained by silver. × 640.

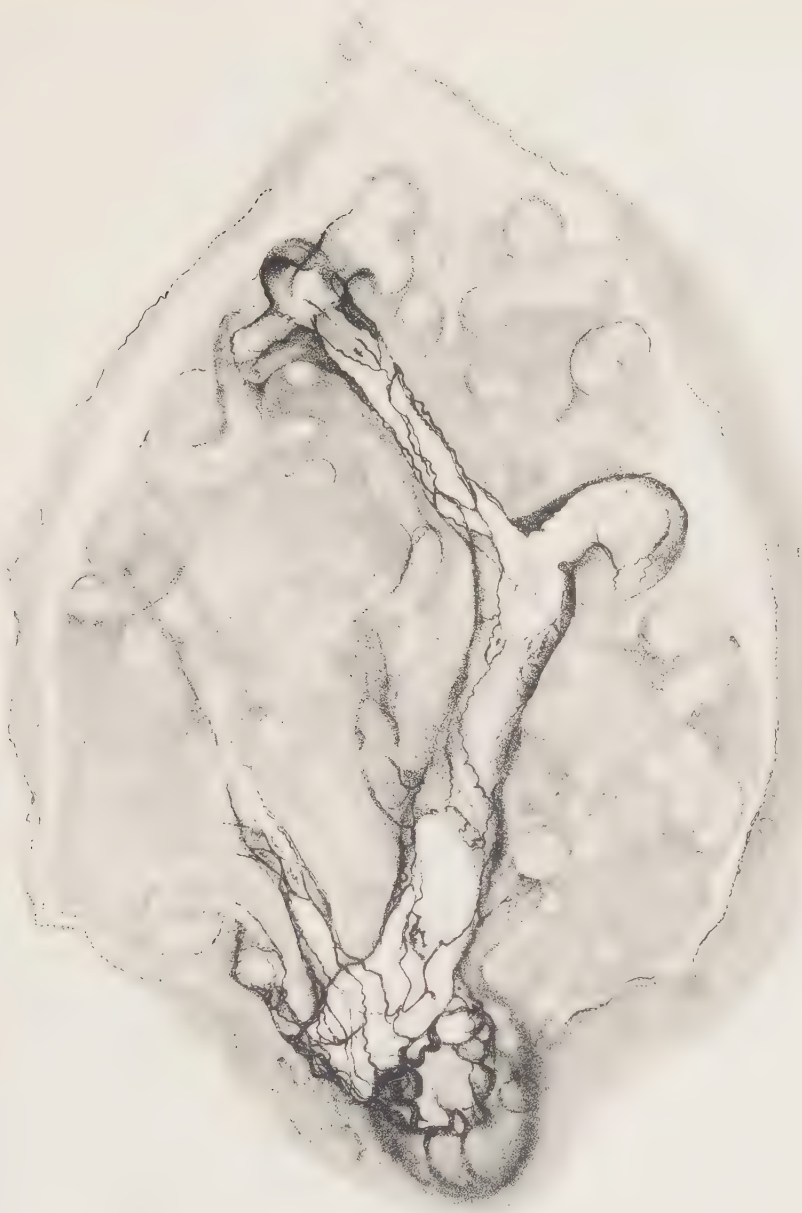


PLATE 4

EXPLANATION OF FIGURE

4 Section of human glomerulus; silver staining for argyrophile fibers. × 640.

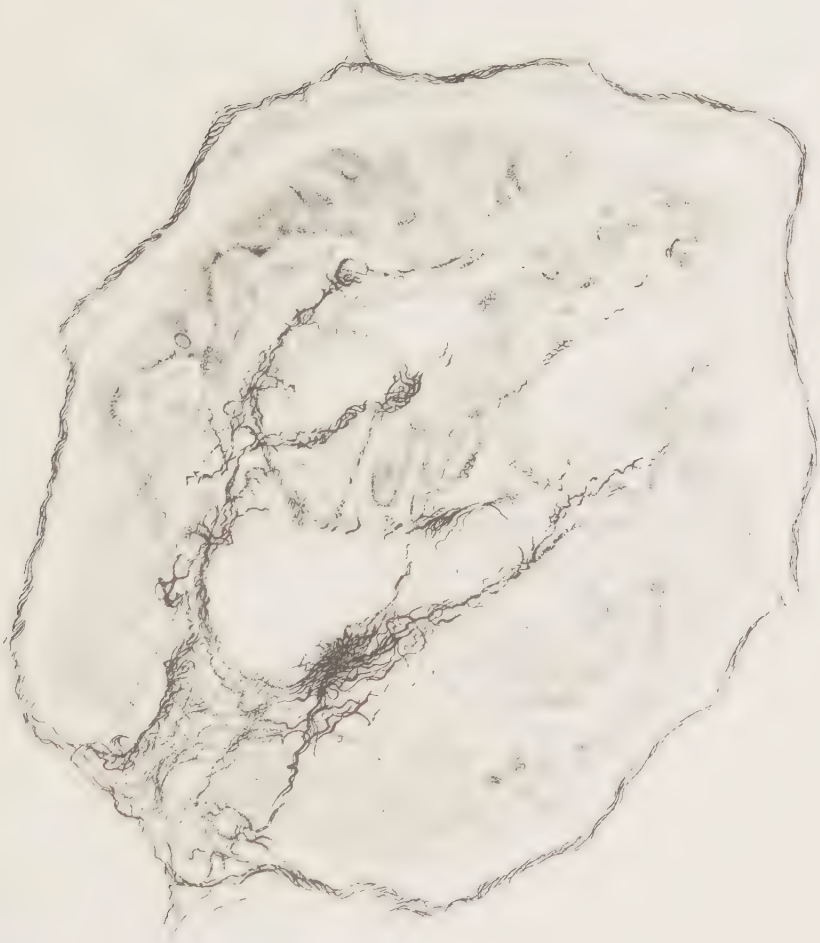
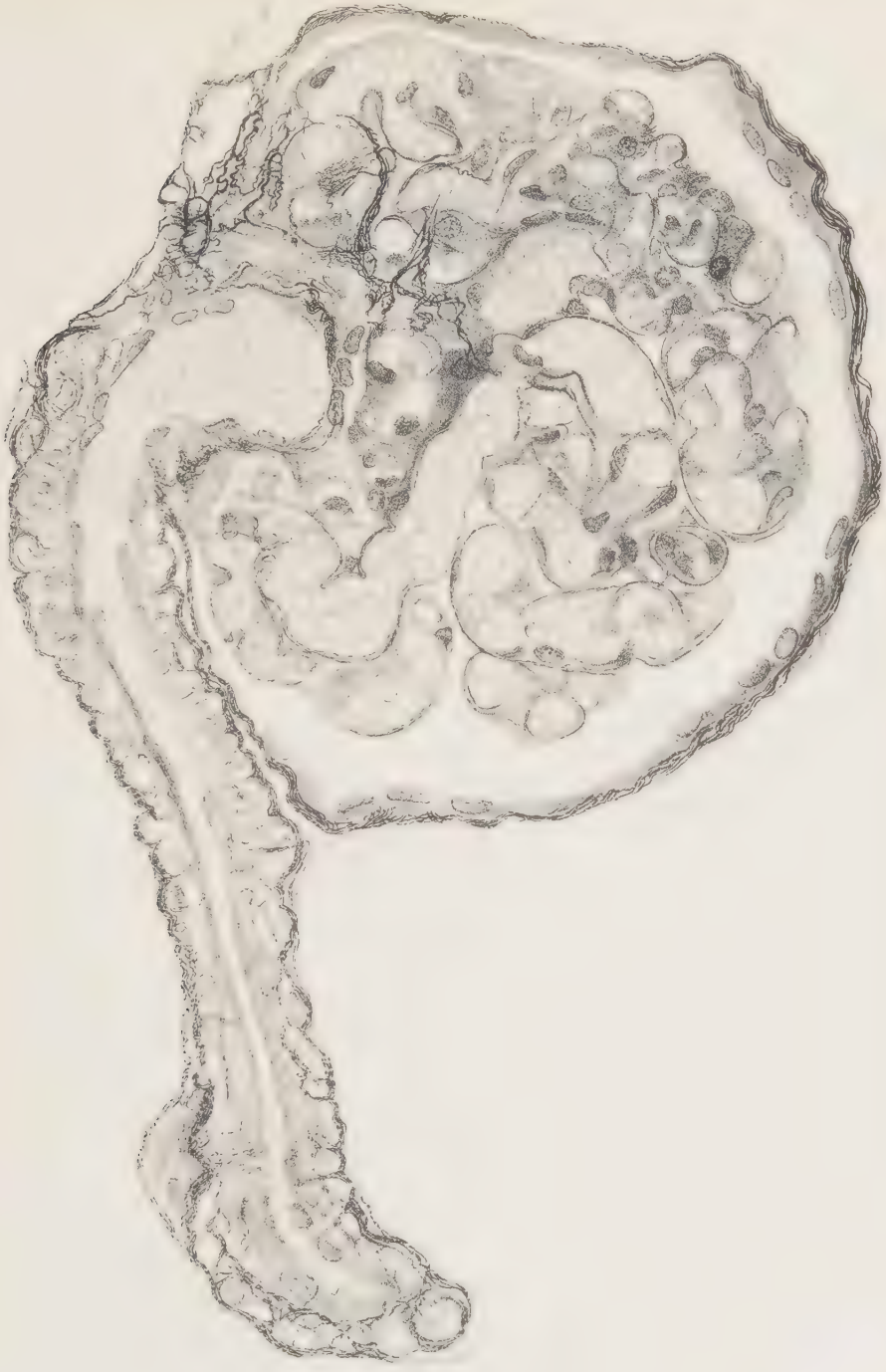


PLATE 5

EXPLANATION OF FIGURE

5 Section of human glomerulus; silver staining for argyrophile fibers and counterstained with acridin red. × 640. This figure shows continuity of argyrophile fibers with adventitia of afferent vessel, and structure of the capsule of Bowman.



STUDIES ON AMPHIBIAN METAMORPHOSIS

VIII. THE RÔLE OF THE UROSTYLE IN THE ATROPHY OF THE TAIL

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ONE FIGURE

INTRODUCTION

The atrophy of the anuran tail during larval involution constitutes a striking example of the complete histolysis of an entire structure and its various tissue components. The general macroscopic features of this degeneration are matters of common observation to students of amphibian metamorphosis, and will therefore be described only briefly here. The structure first becomes narrower dorsoventrally, due to the regression of the integumentary finny portions, and then undergoes a gradual resorption of the muscular tissues with consequent shortening in length. Pigmentary changes likewise occur, and the structure as a whole assumes a darker hue, until eventually only a small, black stump remains which is finally lost as the young frog assumes a terrestrial life.

Before the relationship of the thyroid gland to metamorphosis was suggested by the work of Gudernatsch ('12, '14), several workers had offered explanations regarding the causal mechanism of tail atrophy. Barfurth ('87), as the result of his observations, came to the conclusion that the rapid growth of the urostyle during larval involution tended through pressure to occlude the blood vessels at the base of the tail, and so by interfering with the vascular supply initiated the subsequent atrophy of the entire structure. A few years later, Bataillon ('91), in a study of the vascular supply of the

atrophying anuran tail, concluded that the developing urostyle actually brought about a redistribution of the blood supply throughout the tail. Mercier ('06) later injected carmine granules into the dorsal lymph sac of involuting larvae and demonstrated the subsequent appearance of phagocytes containing carmine granules in the atrophying muscle masses of the tail at a comparatively late stage of metamorphosis. In this manner he was able to show that Barfurth's statement regarding the occlusion of the vascular supply to the tail could only be accepted by assuming that only partial occlusion takes place. More recently, Morse ('18), in a biochemical and histological study of atrophying tail muscle, adopted Barfurth's and Mercier's hypotheses and suggested that the partial occlusion of the vascular supply to the tail results in an accumulation of carbon dioxide and acids of incomplete combustion. This condition, according to Morse, would logically render the reaction of the blood less alkaline or more acid. The possibility that oxygen may normally inhibit the activity of the autolyzing enzymes in the tail and hence that reduction in the oxygen supply through occlusion of the blood vessels might operate to permit autolysis to proceed, was refuted by experiments carried out by Morse. Morse ('18), however, found that no acceleration of tail atrophy followed the ligation of the aorta at the base of the tail and admits that this result does not bear out the theory that interference in the blood supply induces atrophy. He suggests, however, that lateral circulation may have been a factor. The writer ('26) repeated this work and obtained the same result, even after the speed of the capillary blood flow had been reduced considerably. Morse finally suggested that partial interruption of the blood supply everywhere[1] results in an accumulation of carbon dioxide and that the alkalinity of the blood is neutralized and an actual acidity results (pH, 7). As regards the latter point, the writer is at present carrying out work to determine the actual pH of the blood at various stages of metamorphosis.

[1] Presumably, Morse meant everywhere throughout the tail, and not throughout the whole larva.

The above brief review serves to indicate that all workers, without exception, have assumed that the presence of the rapidly growing urostyle was the fundamental primary cause of tail atrophy. It seemed rather unusual to the writer that no one had attempted to remove the urostyle before development had occurred and so either prove or disprove this basic hypothesis. It will be granted, I believe, that if the urostyle is actually the primary cause of tail atrophy, then its removal before differentiation and growth had occurred should result in complete or at least a partial inhibition of autolysis during larval involution. The theoretical possibility of obtaining newly metamorphosed young frogs with larval tails was thereby suggested. In order to determine this point, it was logically necessary to remove the urostyle from normal, non-metamorphosing larvae at a time when the structure is represented as a mere cartilaginous rod or anlage. Preliminary attempts to remove this anlage gave evidence of the difficulty of obtaining successful operations and also, perhaps, a hint as to why this rather apparent and crucial point had not been cleared up in the past. Although the writer has been working for some eight years on various operative problems of amphibian metamorphosis, the successful removal of urostyle anlagen proved to be, by far, the most difficult of any operations attempted. In all, some six different operative approaches and techniques were devised and a total of over 200 larvae operated on before an adequate technique was perfected which would allow of the extirpation of the anlage without subsequent death of the larva or injury to the vascular supply to the tail. The present paper presents the technique finally adopted and the effect on tail atrophy during subsequent metamorphosis.

The writer wishes to express his appreciation for the facilities tendered him during the summers of 1928 and 1929 by the Iowa Lakeside Laboratory, Milford, Iowa, where the experimental part of the present paper was completed.

TECHNIQUE AND RESULTS OF UROSTYLE EXTIRPATION

The larvae used for all operations were obtained from a small pond in the vicinity of Spirit Lake, Iowa, during the summers of 1928 and 1929. This particular strain of Rana pipiens was characterized by exceptionally large larvae, which reached the length of from 110 to 115 mm. before the onset of metamorphosis. When collected and operated on, however, they were but from 75 to 90 mm. in length, with hind legs 6 to 14 mm. long. Such specimens invariably required at least a month to complete their larval growth, and the removal of the urostyle anlage was therefore made at a time considerably prior to any metamorphic changes of the tail or larva as a whole. Prior to the operation, the larvae were well fed for several days with Spirogyra, which treatment enabled them to better withstand the subsequent shock of the operation.

The technique finally found productive of the desired results was as follows: The tadpole was lightly anaesthetized in a 0.05 per cent aqueous solution of chloretone and placed on its left side on a special paraffin operating block. The latter had been previously shaped so as to permit the body of the larva to fit into a depression in the block. The part supporting the tail was so shaped that the anterior portion of the latter was brought into strong flexion and hence elevated in a convex manner, which condition facilitated the operation to be performed. The animal was then fastened immovably to the paraffin block by means of several rubber bands.

The actual details of the operation were as follows: An incision was first made in the integument running from a point somewhat anterior to the dorsal junction of the trunk and tail diagonally down to a midlateral point on the anterior portion of the tail (*SI*, fig. 1). The integument on either side of this incision was then carefully loosened from the underlying musculature and held back by means of several especially prepared hooks connected by threads to pins inserted in the paraffin block. This exposed the musculature of the tail and that of the dorsal side of the back where the

latter unites with the tail. The superficial lateral vein of the tail (*LV*, fig. 1) is now clearly visible, while the various organs of the posterior portion of the abdominal cavity (such as the kidney, *K*, fig. 1) can be seen through the thin peritoneal lining of the latter. Considerable care is essential from this stage on not to injure the peritoneal lining, with consequent extrusion of the abdominal organs which are tightly pressed

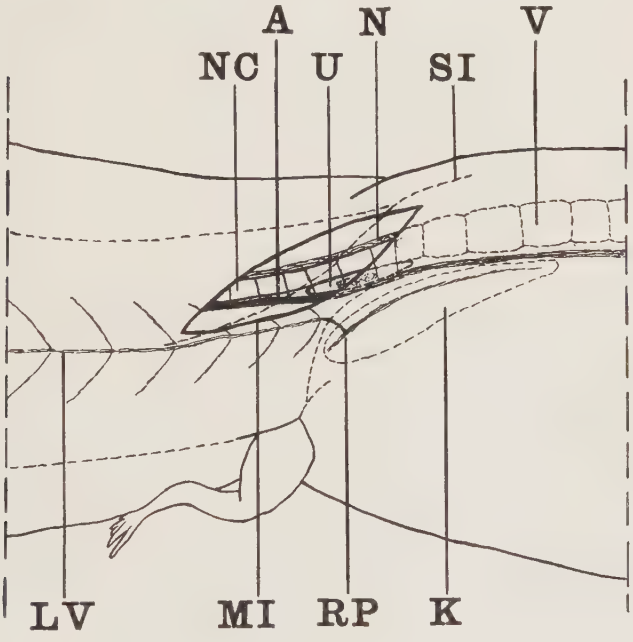

Figure 1

against the lining. Next, an incision (*MI*, fig. 1) is made in the musculature, the exact location of which is very important in that it must be as near the location of the deeply embedded anlage of the urostyle as feasible. This incision is gradually deepened until it is possible to pull and hold the muscular edges apart with hooks. By gradually cutting deeper with a small spearhead needle, the region of the nerve cord, notochord, and dorsal aorta is finally brought to view

(*N*, *NC*, and *A*, fig. 1). At this stage of the operation, the use of a small, strong beam of light is essential to illuminate the field of operation. The musculature immediately surrounding the notochord and aorta is now found strongly adherent to these structures and must be carefully and slowly removed, piece by piece, in order not to injure the aorta especially. By carefully exposing the notochord and dorsal aorta in this manner, the posterior tip of the urostyle anlage is next located (*U*, fig. 1). Several branches of the dorsal aorta are now seen to encircle the anlage and notochord. The anlage is rather difficult to recognize, due to the fact that it is but approximately 2 to 3 mm. in length by 1 mm. in thickness and of the same translucent shade as the adjoining notochord and musculature, to both of which it is strongly adherent. Generally, however, slight signs of ossification (appearing white in color) are evident at about the middle of the anlage which help to locate the structure. It is next necessary to loosen the anlage from the notochord adjoining it dorsally and from the dorsal aorta attached to it ventrally. This is done by carefully cutting the tough membranous connections with the point of a sharp spearhead needle. The separation of the anlage from these structures is made less difficult by gently pulling up the posterior tip of the anlage by means of a slender fine-pointed pair of forceps and gradually severing the connections with the aorta and notochord progressively anterior. It is very easy to puncture the aorta during this procedure and so defeat the purpose of the experiment. The anlage is finally cut free from its attachment with the posterior tip of the vertebral column and removed. The hooks holding the musculature and integument are then removed and the integumentary incision sutured with six or seven stitches of silk thread. It is not necessary to suture the muscle incision, since the walls of the latter spring back and approximate one another, due to the elasticity of the muscle.

Following the removal of the urostyle anlage, the larvae were placed in individual aquaria for subsequent growth and

metamorphosis. Control operations were made which consisted of all steps in the technique as outlined above, with the exception that the anlagen were not removed following their separation from the notochord and dorsal aorta. These controls and others consisting of normal unoperated larvae were maintained along with the urostyle-extirpated animals. All larvae were fed regularly with Spirogyra to promote growth, and daily observations made to detect the first signs of metamorphosis and especially of tail atrophy.

In all, twenty-six cases of urostyle extirpation and eleven operated controls were obtained which survived the operation and lived through subsequent metamorphosis. The muscle and skin incisions were invariably fully healed within one week following the operations. Due to the precaution of selecting larvae of the same developmental stage, the onset of metamorphosis was found to occur between five and six weeks following the date of operation. In both types of control animals and in the urostyle-extirpated individuals, the resorption of the tail was found to take place in a normal manner. There were no cases of delayed tail atrophy in either the operated controls or the urostyle-extirpated larvae, as compared with the metamorphosing normal controls. Following the complete resorption of the tail and the end of metamorphosis in general, the urostyle-extirpated individuals were autopsied, in order to determine whether regeneration of the urostyle anlagen had occurred. In no case could such regeneration be detected, the inference being that the anlagen had been completely extirpated at the time of operation.

DISCUSSION

The results of the present work indicate that the explanation of Barfurth ('87) regarding the growth of the urostyle as the fundamental cause of anuran tail atrophy and similar hypotheses, as proposed by Bataillon ('91), Mercier ('06), Morse ('18), and Bradley ('22), are no longer tenable. Even in view of the fact that the developing urostyle no doubt does function to compress the dorsal aorta, the results of

urostyle extirpation which prevented occlusion of the aorta by pressure and those of Morse ('18), and the writer ('26) on ligation of the aorta in normal larvae, clearly indicate that occlusion of the aortic blood vessel is not necessary or causative for the normal atrophic processes which occur. Furthermore, there is no evidence at present which relates the normal partial occlusion of the aorta with the rearrangement of the peripheral blood vessels and capillaries as described by Bataillon ('91). The writer is more inclined to believe that vascular rearrangement and reduced blood flow through the tail are the result, and not the cause, of the various histolytic changes which occur during larval transformation.

The results of the present work were strikingly supplemented by the simultaneous findings of Lindeman ('29). In the course of work involving reciprocal, autoplastic back- and tail-skin transplantations, the latter worker demonstrated that tail skin transplanted to the back underwent typical histolysis during metamorphosis at the same time the integument of the tail was undergoing degeneration. The degeneration of the grafts in a foreign location, removed from any possible influence of the developing urostyle, suggested the possibility that the factors responsible for tail-skin histolysis must be chemical ones transported through the blood stream. Furthermore, the fact that back-skin grafts transplanted to the tail failed to undergo degeneration during the subsequent complete atrophy of the latter structure was taken as evidence that tail skin is peculiarly specific and different from other integument in its susceptibility to certain histolytic agents present in the blood stream. Later, Helff and Clausen ('29) were able to show somewhat similar results with reciprocal, autoplastic tail-muscle transplantations. It was shown in this work that whereas tail muscle transplanted to the back underwent complete degeneration and obliteration during metamorphosis, back muscle previously transplanted to the tail atrophied but 54 per cent during the same time interval. Again, this would indicate that the musculature of the tail is especially susceptible to certain histolytic agents of the

blood stream. Incidentally, Clausen ('29) has been able to show that a gradient in histolytic susceptibility is present, in that skin and muscle grafts taken from anterior regions of the tail and transplanted to the back undergo more rapid histolysis during metamorphosis than is true of similar grafts obtained from more posterior regions of the tail.

The results of the present paper, coupled with those of Lindeman ('29), Helff and Clausen ('29), and Clausen ('29), therefore strongly indicate that the various tissues of the larval anuran's tail inherit a susceptibility to certain histolytic agents which are liberated and become functional at a certain definite stage of metamorphosis. As yet, the exact nature of these agents is quite problematic. It is quite possible that a general lowering of the blood pH may be the fundamental factor operating to activate the autolytic enzymes present in the tissues of the tail. It is also quite possible that specific chemical substances may be liberated to the blood stream, through the degeneration of other larval organs, which are capable of inducing histolysis of the tissues of the tail. The possibility that the thyroid hormone acts directly through the blood stream to initiate tail atrophy seems rather unlikely, since the tissues of the tail often remain quite normal during the early stages of metamorphosis and at a time when profound developmental and degenerative processes are occurring elsewhere in the larva.

SUMMARY AND CONCLUSIONS

1. The investigations of Barfurth, Bataillon, Mercier, and W. Morse have tended toward the assumption that atrophy of the anuran tail during metamorphosis is due primarily to urostyle development. This rapidly developing organ, by partially occluding the dorsal aorta, was thought to result in acidosis of the tissues of the tail, with resultant autolysis.

2. The urostyle of the adult develops from a small cartilaginous rod, 2 to 3 mm. in length, located near the junction of the body and tail between the notochord and dorsal aorta. This anlage is difficult to remove, but a successful method of

extirpation was finally developed. Twenty-six cases of extirpation were obtained which lived through subsequent metamorphosis. It was found that normal tail atrophy took place during involution simultaneously with groups of normal and operated controls. Subsequent autopsy failed to reveal regeneration of urostyle cartilage. It is concluded that the growth of the urostyle cannot be considered the fundamental cause of tail atrophy, while the tissues of the tail appear to be specifically susceptible to histolytic agents in the blood stream.

3. The results are discussed in general and in relation to other recent work on tail atrophy. Possible lowering of the blood pH and the presence of specific chemical substances capable of inducing histolysis are suggested as possible causative factors.

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RECENT MODIFICATIONS IN THE METHOD OF STUDYING LIVING CELLS AND TISSUES IN TRANSPARENT CHAMBERS INSERTED IN THE RABBIT'S EAR

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EIGHT FIGURES

Studies of the morphological characteristics, mode of growth, and reactive powers of living cells and tissues in the living animal have been carried out by one of the authors since 1908. For most of these studies the transparent tail fin of amphibian larvae was used as the object of study, since it was possible there to carry out long-continued observations with highest magnifications of the same vessels and cells in their normal environment (E. R. Clark, '09, '12, '18). Although the tadpole's tail affords an ideal object for such studies, it was not certain to how great a degree the results obtained were applicable to higher animals, and hence it seemed advisable to carry out similar studies in the living mammal.

Since no natural transparent region, adapted to intensive high-power observation in the living over long periods of time, was available in the mammal, it was necessary to create a method for obtaining such a region, and the plan of inserting a transparent chamber in the rabbit's ear was devised.

Much of the pioneer work in developing this technique was carried out by J. C. Sandison in this laboratory, and descriptions of the earlier methods have been published (Sandison, '24, '28 a). In 1927, Sandison succeeded in obtaining new growth of tissue, including blood vessels, in chambers made

of kodaloid (celluloid), and in portions of such chambers the tissue was sufficiently thin to permit the study of blood vessels, blood cells, etc., with the oil-immersion lens (Sandison, '28 a and b). Sandison ('28 b) also obtained a new growth of bone, transplanted into such a chamber at the time of its insertion. Recently, H. C. Hou ('30), of Peiping University, who worked in this laboratory with the method in 1928, has obtained transplants of adrenal tissue in a chamber in the rabbit's ear which remained alive for several weeks.

The method as developed from 1924 to 1928 made possible the only means available up to that time for long-continued studies of mammalian living cells and tissues with high magnification, and some studies were carried out successfully; however, the method still needed perfecting. The chief defects to be overcome before the method could be available for general use with the assurance that observations could be made with any degree of certainty were: lack of permanence of the chambers; the difficulty of obtaining a space sufficiently thin for study of a large area of tissue with the oil-immersion lens; and the length of time necessary for the new growth of tissue to reach a thin space where it could be studied to best advantage. The earlier chambers remained in the ear for four months at the longest, while many of them came out at the end of two or three weeks. Moreover, although the earlier chambers were planned and constructed so as to contain a space thin enough for high-power study, the actual retention of such a space was a matter of great uncertainty, owing to the marked tendency of the thin kodaloid cover to warp. And lastly, although growth started soon after the operation, the time necessary for the new vessels and tissue to reach the thin spaces where careful observations could be made was frequently so long that the chamber might become infected or might drop out of the ear before such studies could be carried out.

For the past year and a half (since the fall of 1928), a number of workers in this laboratory have been engaged in the investigation of problems which involved the use of the

method, and several of this number have devoted a considerable amount of time to its improvement. Most of the difficulties inherent in the earlier technique have now been overcome and, in addition, new modifications have been worked out which permit the use of the chambers for a wide variety of experiments as well as for more satisfactory growth studies. In addition, an entirely new method has been developed which permits the study of preformed tissue in transparent chambers and a comparison of the original vessels and tissue with the new vessels and tissue which grow into the chambers.

It seems unnecessary to recount the many steps in the process of improving the chambers or to describe the different types which have been inserted during this period of experimentation. Each change in construction or insertion of the chambers was made in order to meet one of the difficulties already mentioned. In many of the chambers thus inserted extensive growth of new tissue was obtained; however, experiments were continued until types of chambers were developed which were satisfactory in all respects.

In order to obtain permanency, the first procedure was to alter the shape of the chambers, since it was found that the sharp, square corners of the earlier types sooner or later invariably cut through the skin. To obviate this difficulty, only round or oval chambers are now used. Drying and retraction of the cut edges of the skin, which always occurred after the earlier type of operation and added to the insecurity of the chamber, were prevented by the use of protective collars of heavy kodaloid. The operative technique was also altered so as to include more of the cartilage of the ear within the chamber—a procedure which added greatly to the strength and stability of the chambers. All of the chambers now in use are bolted through the ear at the time of operation with small bolts and nuts (a re-introduction of an earlier procedure (Sandison, '24)). The tendency of rabbits to scratch their ears—which formerly hastened the process of extrusion of a chamber already loosened—has been practically eliminated by the simple process of freeing the animals from fleas.

In our efforts to obtain a uniformly thin space for study with the oil-immersion lens, a great many experiments were carried out. One of the first improvements consisted in the substitution of ether and alcohol (absolute, anhydrous 50-50) for sealing parts of the kodaloid chambers together, instead of the parlodion formerly employed. The ether-alcohol combination softens the surfaces of the kodaloid and the resulting union of the two pieces becomes an organic one. For the more delicate parts of the chamber the sealing is carried out under the binocular dissecting microscope, the ether-alcohol mixture being blown into the desired place through a small glass cannula. In other cases the mixture is poured out in the lid of a Stender dish, the small kodaloid tables or discs picked up in fine forceps, one side dipped into the ether-alcohol and transferred quickly to the larger piece of kodaloid, to which it is to be fastened. The ether-alcohol combination makes possible the obtaining of clearer observation tables free from bubbles, a much firmer union between parts of the chamber, and helps to eliminate warping of the kodaloid. However, warping of the thin kodaloid used for the top of the chamber through which the observations are carried out still occurred. To overcome this defect, the architecture of the chamber was radically changed.

The 'bay' chamber is the most successful result of our efforts to obtain a uniformly thin space with the use of the thin kodaloid top. However, after inserting various chambers constructed with an extensive thin space, a good deal of difficulty was encountered on account of drying. This we found to be due to the permeability of the thin kodaloid to moisture. The serum always present in such thin spaces before the new tissue and capillaries had grown out into them frequently dried out soon after the operation and was transformed into a thick glue, through which the growing capillaries were unable to penetrate.

This loss of fluid from the chambers—particularly disastrous in the first week after the operation—was prevented in two ways: first, when it seemed necessary to use koda-

loid, by sealing a removable, moist compartment, containing a piece of gauze or absorbent cotton soaked with fluid, next the thin kodaloid cover on the outside, and, secondly, by abandoning the use of the thin kodaloid cover altogether and substituting thin mica for the top.[1]

The third defect—the length of time elapsing between the operation and the period at which observations on new vessels with high magnification can be made—was remedied by including more of the original cartilage and blood vessels in the periphery of the chambers and by decreasing the distance between this tissue and the thin space designed for high-power microscopic study. In the chamber in which preformed tissue is retained, observations can be started immediately after the operation.

The first and second types of chambers which will be described are models designed for high-power study of the new growth of vessels and tissues. They successfully meet the difficulties inherent in the earlier method. These chambers have also been adapted for various experiments, especially for the transplantation of pieces of organs and tissues from other non-transparent regions of the body and for the introduction of various foreign substances. The third type of chamber is an entirely new one, designed for the retention of *preformed* tissue, especially of blood vessels, in a space sufficiently thin and transparent to permit of careful study. And the last type of chamber described here is a combination of the last two methods which was designed in order to make possible a careful morphological and physiological comparison of preformed blood vessels with new-growing ones in the same ear.

THE 'BAY' CHAMBER

This type of chamber in the form first designed was demonstrated at the Rochester session of the American Association

[1] Mica had been used in some of the early chambers (Sandison, '24) and abandoned for the thin kodaloid, on account of its propensity for splitting and the ease with which it becomes scratched. These difficulties have been largely overcome by the improved techniques, and for most purposes the thin mica cover is more satisfactory than the thin kodaloid.

of Anatomists (E. R. Clark, S. A. Matthews, and H. T. Kirby-Smith, '29). The collars of heavy kodaloid which projected outward, enclosing and protecting the cut edges of the skin on both sides, gave this chamber the feature of permanency. The thin spaces for observation were obtained by making three small depressed areas for growth ('bays') in the kodaloid bottom, instead of the elevated tables of the earlier chambers. These bays are very shallow (about 0.75 μ deep) and communicate through deep tunnels with the groove made for the artery. The thin kodaloid cover could then be fastened down to the bottom with ether-alcohol everywhere except over the small span occupied by the depressed bays, and much of the warping was thereby avoided. The loss of moisture through the kodaloid cover was counteracted by keeping a moist cotton or gauze pledget in an outer chamber which was sealed to the outer collar and removed for the daily observations. During long-continued observation periods, evaporation of fluid from the thin areas of growth was prevented to some extent by keeping a film of paraffin oil on the thin kodaloid cover.[2]

In this original form of the 'bay' chamber very thin areas of growth were obtained. However, after a month or more, the new tissue showed a tendency to retract from the bays. To prevent this retraction, the bays were extended and joined by connecting passages, in the hope that the new tissue would grow through these tunnels and unite in the bay, thus providing an anchorage for the growth.

The bay chamber as now used is constructed as follows:

1. An oval piece of kodaloid (Eastman extra heavy) is used for the bottom and is bent at one side to make a groove for the main artery, as in the older type of chamber (fig. 1, *6*).

[2] A number of experiments were carried out with different shellacs, water glass, etc., to obtain a water-proof coating for the thin kodaloid covers. A coating of valspar varnish best passed the test of transparency and insolubility in moisture before insertion of the chambers, but chambers prepared with a thin kodaloid cover coated with valspar after insertion in the ear showed a checking of the varnish and in addition failed to prevent loss of moisture from the new-growing tissue.

2. Two rectangular holes (for the tunnels) are cut in a piece of extra heavy kodaloid, figure 1, *5*, so placed as to lie over the bend in the bottom piece. This piece is then fastened to the heavy bottom with ether-alcohol.

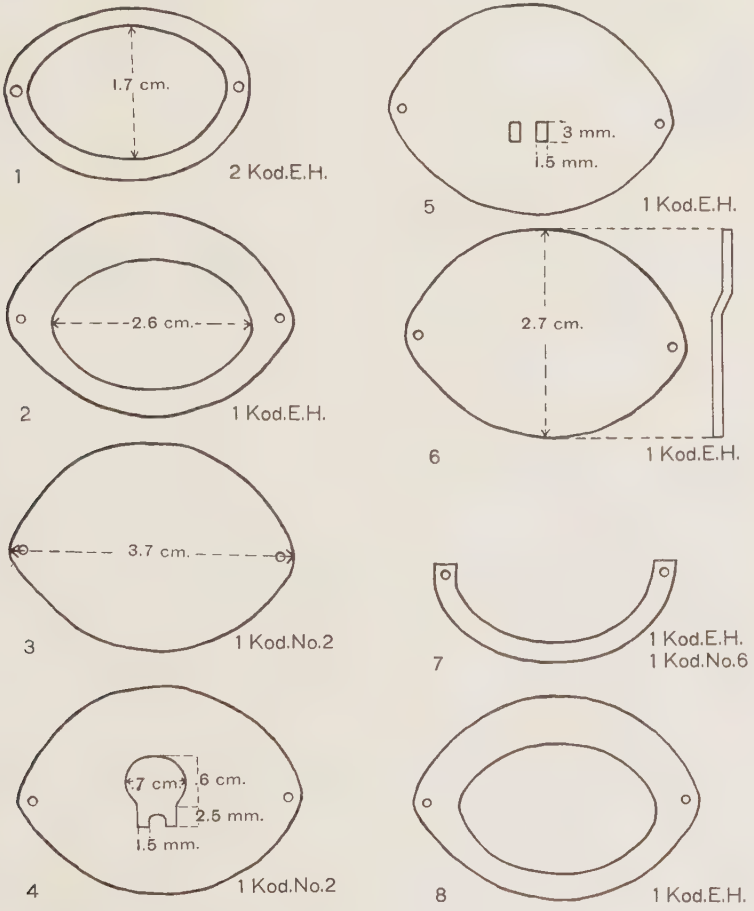

Fig. 1 Diagram showing the dimensions, parts, and materials used in the construction of the 'bay-type' chamber. The diagrams from 1 to 8, inclusive, represent the parts in the order of their appearance in the finished product from above downward. On the bottom of the chamber there are two pieces similar to those seen in diagram 1 which are not shown in the figure. The numbers of pieces and the material are given at the right of each figure. *Kod.* refers to kodaloid, of which three thicknesses are used: *E.H.*, extra heavy, no. 2, and no. 6.

3. Two rectangular holes are now cut in a piece of no. 2 (thin) kodaloid so that they overlap the tunnels cut in the bottom almost completely. These holes are continued to form the bay, as shown in figure 1, *4*. This no. 2 sheet is fastened down to the bottom with the rectangular holes overlapping the cuts in *5*, figure 1.

4. A second piece of no. 2 kodaloid is now fastened down to the others with ether-alcohol to form a cover for the bay (fig. 1, *3*).

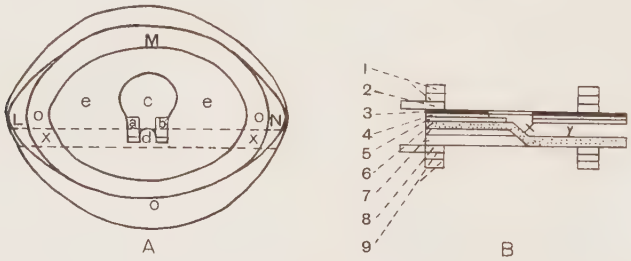

Fig. 2 A. A diagram of the assembled 'bay-type' chamber as seen from above. *a* and *b* are tunnels into which the tissue grows and unites in the bay, *c*, thereby helping to hold the chamber in place by surrounding the post at *d*. *e* is solid, being formed by the gluing together of the overlying pieces as described in the text. *x* marks the bend in the base in which the main artery of the ear lies. B. A diagram of the 'bay-type' chamber as seen in a section across the narrow axis at *o* in diagram A. The numbers refer to corresponding numbers designating the separate pieces shown in figure 1. The heavy kodaloid rings are not shown in figures 1 or 2, since they are retaining rings, and not a part of the chamber.

5. Two rings of (Eastman extra heavy) kodaloid set in about 2 mm. from the outer edge of the chamber are glued to the top to make a ledge for the skin (fig. 1, *2*).

6. A partial ring of extra-heavy kodaloid and one of no. 6 kodaloid (fig. 1, *7*) are glued to the base as indicated at *7* in figure 2B. This is to compensate for the bend in the base. Then two sets of rings composed of two pieces similar to *1*, figure 1, are glued together and attached to the chamber at *1* and *9*, figure 2B. Two holes—one on each end—are burned with a needle through the rings from top to bottom. These are used for screw holes.

7. Two protective collars are cut from Eastman extra-heavy kodaloid with an opening for the central part of the chamber (inside the rings) and two holes burnt in each to correspond to those through the rings on the chamber proper. These are left detached until the time of operation.

For the insertion of this type of chamber, the preliminary operative procedures are the same as for the other chambers described here and similar to those given in the original description of the method. Rigid aseptic technique is used in the operating room. The ear is prepared by first clipping the hair and then carefully shaving the distal two-thirds on both sides. The ear is then immersed in a glass of 1 per cent phenol for five minutes and afterward rinsed with sterile Ringer's solution. In some cases, a wet dressing soaked in 1-to-2500 metaphen has been substituted for the phenol. Since the rabbit's ear is tender and the skin easily injured, strong chemical disinfectants and depilatories cannot be used. Cocaine or novocaine anaesthesia is used. The chamber is sterilized in hexyl-resorcinol (full strength) or metaphen (1 to 500). After sterilization, it is washed thoroughly with sterile distilled water.

To insert the chamber, one proceeds as follows: The site is selected so that the central artery of the ear will lie at the bend in the base of the chamber. With the back of a scalpel, the skin to be excised is marked on the inner surface of the ear. This mark takes the shape of the line *L M N O* in figure 2A. The skin is then incised in the long axis and raised by blunt dissection to the line just marked, where it is cut off with scissors. A hole is now to be made in the ear which will have the shape represented by the lines *L M N* in figure 2A, the straight cut being placed so that it comes 1 mm. from the artery. The skin on the outer surface of the ear is elevated and removed in a manner corresponding to that employed on the inner surface. One now has a projecting portion of cartilage carrying the principal artery. The cartilage will occupy the position *X-Y* in figure 2B, the artery lying at *X*.

The chamber is inserted with the thin kodaloid top on the inner side of the ear, in order to facilitate observation, since the natural fold in the ear makes it difficult to keep the ear flat with the dorsal side uppermost. The chamber is first slipped under the skin on the outer side of the ear and, as this is done, the cartilage is caught in the space *X-Y*, figure 2B, and the chamber gently forced into place. The skin is thus fitted well up against the elevated rings on both sides. Finally, the two outer collars are fastened on with two bolts. A strip of sterile gauze is inserted under the collars to absorb the serum which oozes out around the edge during the first days after the operation. A small piece of cotton soaked in sterile water is placed on top of the thin kodaloid inner side and a piece of no. 10 kodaloid, cut the size of the outer collar, sealed over it with wax-and-resin mixture. This outer chamber is removed for daily observation, the cotton remoistened, and protective collars replaced.

This type of chamber is especially valuable for long-continued studies of blood capillaries which are kept free from pressure and movement of the surrounding tissue. It is very lasting—one such chamber inserted nine months ago is still in the ear, and shows no signs of being extruded. Some modifications of this type of chamber are now being worked out in which an extra tunnel from the side is constructed leading into the small rounded end of the bay, into which a tube may be inserted for the purpose of submitting the vessels and cells to the action of known chemical solutions.

THE ROUND-TABLE CHAMBER

This chamber, which has been in the process of development for the past year, has proved to be the most satisfactory one used so far for the study of new growth of tissues with high magnifications. It is now standardized so that some of the parts can be cut out mechanically and chambers of uniform size made by a technician.

This chamber is in part a return to an older type tried and abandoned by Sandison ('24), in that for the top of the chamber, or the observation side, it involves the substitution

of thin mica for the no. 2 kodaloid. These chambers are fastened together in the ear at the time of operation by means of nuts and bolts. The construction of the present variety, however, has been modified so as to overcome the disadvantages of the earlier form, while at the same time the tendency of the thin kodaloid to warp and to allow the escape of fluid is avoided. The use of viscoloid—a heavier form of celluloid—instead of the Eastman extra-heavy kodaloid for the bottom of the chamber insures a firm, stable base, since even the bottoms of the older forms of chambers warped after being subjected to the moisture of the tissues. Viscoloid, however, contains many small black specks absent in the kodaloid, and for that reason is used only for the supporting portions of the chamber, pieces of kodaloid cemented together being used for regions of observation.

This chamber is constructed as follows:

1. A hole is cut in the center of the viscoloid bottom with a no. $\frac{1}{16}$ inch bit in order to permit the passage of more light to the region in which observations are to be made.

2. A sheet of thin, clear kodaloid (Eastman no. 6) is then cut the size of the bottom and fastened down to the viscoloid with ether-alcohol, care being taken not to allow any of this mixture to touch the center. A piece of no. 2 (thin) kodaloid is cut in the same shape as the base, to the opposite side of which it is glued for the purpose of counteracting any tendency to warp.

3. Two discs of Eastman extra-heavy kodaloid, especially selected for clearness, are cut out just the size of the central hole and stuck together under the binocular dissecting microscope, care being taken to avoid including any dust or air bubbles between them, and the resulting round table put in a press between glass slides for several hours. The table is then beveled around the edge and glued with ether-alcohol to the no. 6 kodaloid over the central opening.

4. Three round kodaloid washers are stuck to the heavy viscoloid rim of the bottom, equidistant from each other and from the central disc, and a hole burnt in the center of each for the insertion of the screws.

5. The top is composed of a circle of heavy viscoloid the same size as the bottom, with an opening cut in the center with a 5/16-inch bit. This opening is beveled on one side to allow room for the objectives of the microscope. On the opposite side a disc of mica, 0.003 inch thick and especially selected for clearness and absence of scratches, is glued to the kodaloid with a transparent colorless mixture invented by B. B. Varian, of this department (described separately). Holes corresponding to those in the kodaloid washers of the bottom are burnt with a hot needle. Silver has also been used for making the ring for the top of the chamber by pounding out a ten-cent piece on an anvil. In this case the mica is fastened to the silver with very thick balsam heated until it bubbles.

6. The last procedure before the chamber is ready for insertion consists in the gluing on around the edges of the central table of four minute celluloid 'buffers' approximately 1 to 2 mm. square and 40 to 50 μ in thickness. Both the cutting and gluing of these small buffers are carried out under the binocular dissecting microscope. For a graphic explanation of the construction of this chamber, see figure 3.

This type of chamber may be inserted either with the top on the inner or on the outer surface of the ear. The former, however, is preferable for the same reason mentioned in the case of the 'bay' chamber—greater ease of observation—and also for greater protection of the mica cover.

At the time of operation the site of inserting the chamber is carefully selected with regard both to the position of the main artery (which is included at one side of the chamber) and with reference to the holes which are to be cut for the insertion of the central table and the three screws.

A small incision 1 cm. long is made through the skin on the outer side. The skin is lifted carefully from the underlying tissue with a flat blunt dissector, especially made for the purpose, over a circular area corresponding to the line *XY* in figure 3. This is done carefully, so as to leave as many uninjured blood vessels as possible adherent to the cartilage. This skin is then cut away with scissors.

Next, the ear is turned over and the skin of the other side treated in the same manner. The base of the chamber is then slipped under the skin on the outer side, with the central table toward the denuded surface. The ear is again turned over, and screw holes just large enough to fit around the

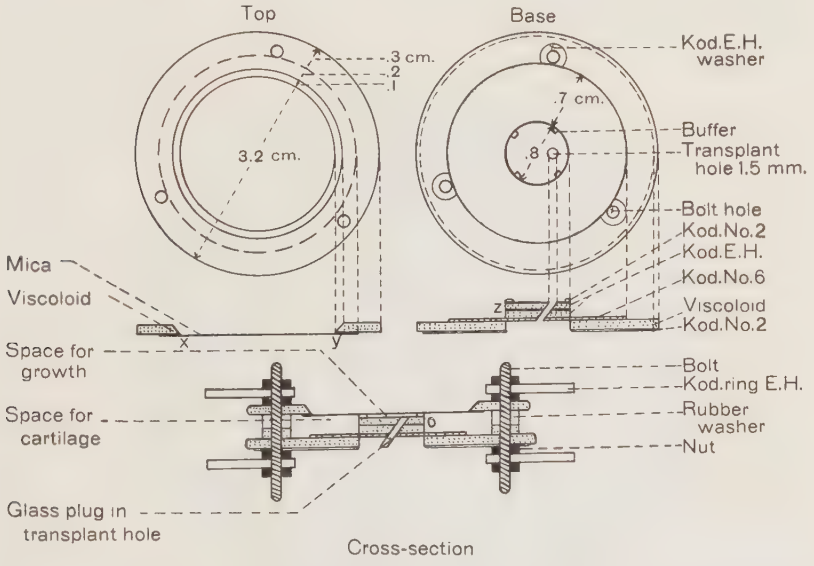

Fig. 3 Diagrams showing the dimensions, parts, and materials used in the construction of the round-table chamber. *Kod.* refers to kodaloid, an Eastman product. This is used in various thicknesses: no. 2, 40 to 75 μ thick; no. 6, 0.24 mm. thick; *E.H.*, extra heavy, 0.62 mm. thick. The viscoloid is 1 mm. thick. The bolts and nuts are of brass, the bolts being made from no. 16 gauge wire, the nuts from octagonal brass rods. The mica used for the top is 60 to 100 μ thick. Once the chamber is inserted, it is protected and supported by two extra-heavy ring collars cut to fit as shown in the cross-section. The hole and plug used in transplanting tissues into the chamber may be omitted.

kodaloid washers are cut through the ear with knife and scissors. Finally, a central hole is cut in the cartilage, so that the table *Z* in figure 3 will fit in, allowing the artery to lie at *O* in figure 3. Three headless bolts with three nuts screwed partway down (used instead of heads) are then pushed through the three holes, and rubber washers, cut to fit the kodaloid washers, are started on them.

The top of the chamber is then fitted on the screws and three nuts screwed down on it. This part of the operation is carried out with the aid of a binocular loupe, in order to observe when the top of the chamber is screwed down tight enough to meet the kodaloid buffers on the central table, thus insuring a uniformly thin space. Solid protective discs of kodaloid are placed on each side of the ear with nuts to hold them down. A single layer of sterile gauze is tucked around under the outer collars. The gauze is renewed, if necessary, during the first few days following the operation. After a week, it can be removed permanently.

Six to eight days after the operation, new capillaries begin to grow out onto the central table, and at this time the two outer kodaloid protection discs are removed and round holes cut in the center to permit of easy observation. They are then replaced as before, after which they serve as protecting collars for the skin at the edges of the chamber.

This chamber may be so modified as to permit the introduction of pieces of organs and tissue onto the observation table at any period of the chamber's life, even after the new blood capillaries have grown across the tables. For use in transplant experiments of this kind, a hole is made with a dental drill through the bottom of the central table—leading obliquely up onto the growth space—and the hole filled with a glass plug and sealed at the bottom with a small drop of paraffin (fig. 3).

Figure 4 is a photograph of the new blood vessels on the central table of such a chamber. The results obtained in this type of chamber are now so uniform that it is possible to prophesy within a few days the time required for new vessels to start growing onto the table and the time necessary for them to cover it.

THE 'PREFORMED-TISSUE' CHAMBER

This type of chamber was developed recently for the purpose of obtaining a thin transparent area in which preformed vessels with their original nerve supply could be studied and

compared with new-growing vessels. This method is especially valuable for physiological studies of circulation under normal and experimental conditions. It also has the advantage of being available for study immediately after the operation. The principle of the preformed-tissue chamber consists in the retention of a layer of skin with all its blood vessels, nerves, and other tissue and the substitution of a

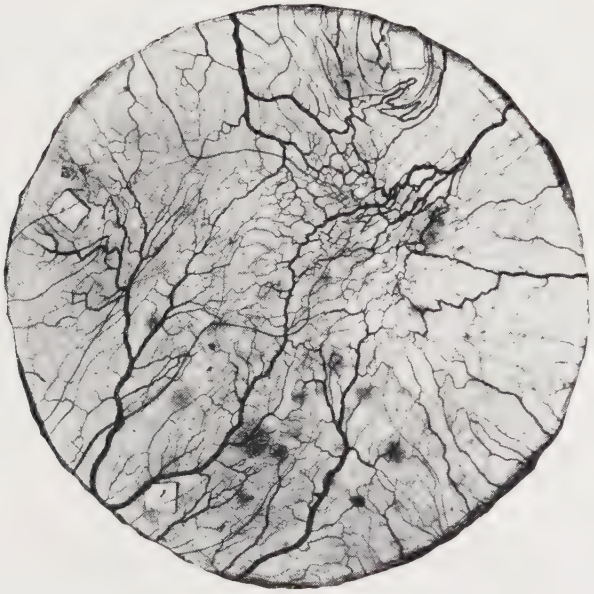

Fig. 4 Photomicrograph showing the character of the blood vessels in a 'round-table' chamber. The photograph shows the entire area of the central table. All of the vessels shown are new-formed blood vessels whose daily growth was watched and recorded. Photograph taken twenty-six days after the insertion of the chamber; growth of new vessels at the edge of the table started at seven days. × 12.

thin mica covering for the cartilage and skin of the other side. The thinness of the retained tissue is maintained by pressure exerted on both sides.

The construction of this chamber is as follows:

1. The bottom or observation side of the chamber consists of a disc cut out of heavy viscoloid with a bit. The outside diameter of the disc is shown in figure 5. A hole is made in the center with a $^{12}/_{16}$-inch bit.

2. Two narrower rings are then cut out from the heavy viscoloid with bits, one with an $^{11}/_{16}$-inch and one with a $^{10}/_{16}$-inch opening. These are glued with ether and alcohol over the $^{12}/_{16}$-inch hole in sequence $^{10}/_{16}$, $^{11}/_{16}$, $^{12}/_{16}$. These are put in a press overnight.

3. The edges of the chamber both at the periphery and around the central opening are then beveled on the one side so as to make an even slope to accommodate the microscope objective. Three round viscoloid washers, made of two thicknesses of double extra-heavy kodaloid, are cut with a punch. These are glued on to the outside ring of the base at equal distances from each other.

4. A round cover of clear mica (0.003 inch thick) is cut the size of the outside diameter of the smallest ring and glued to it with the special glue already mentioned. A *slight* excess around the edge serves to prevent the mica from splitting.

5. The top (side opposite from that used for observation) is made of a viscoloid ring with $^{12}/_{16}$-inch-bit opening. To this is glued a circle of heavier mica, 0.004 inch thick, cut sufficiently large to overlap the central opening. Holes are burnt in the viscoloid ring to correspond with those in the washers of the opposite side (fig. 5).

The technique of operation, the preparation of the ear, anaesthesia, etc., are the same as with the other types. The ear is shaved very carefully on both sides. The bottom of the chamber is placed carefully on the inner side of the ear in the proper position to include the main artery at one side of the table and to avoid striking large vessels at the places to be cut for the insertion of the screws. It is then pressed down so that the table leaves an impression on the skin. This ring is marked with a knife blade. The skin over the marked area is then dissected away very carefully from the cartilage, using a probe and a blunt dissector. After this, a small slit is cut in the cartilage and this dissected away carefully from the skin and blood vessels beneath. The exposed area is kept moist by dropping sterile Ringer's from a pipette at frequent intervals during the operation. The circle

made in this manner through skin and cartilage must be an exact fit for the outer edge of the elevated part of the base.

The chamber is then fitted into the opening and holes punched through the ear with needles thrust through the holes in the three viscoloid washers to mark their location. The chamber is removed temporarily and three holes punched

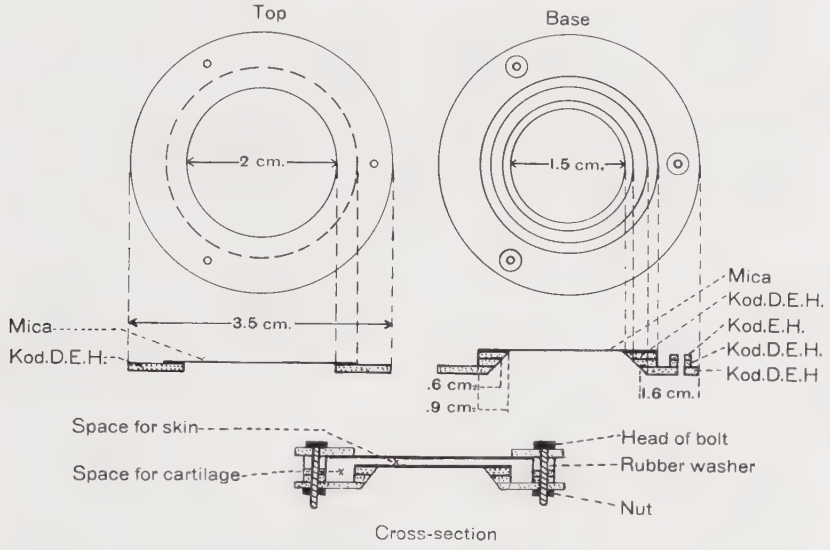

Fig. 5 Diagram showing the dimensions, parts, and materials used in the construction of the preformed-tissue type of chamber. *Kod.* refers to kodaloid (celluloid). *D.E.H.*, double extra heavy; *E.H.*, extra heavy. The bolts differ from those used in the previous chambers in that they have fixed heads. Observations are made through the base, since this part is in direct contact with the original vessels of the ear. The tension on the included skin is regulated against the rubber washer.

at these locations with a leather punch the same size as the round viscoloid washers. The inside of the mica and the exposed area are washed carefully with sterile Ringer's, and bolts with heads inserted in the three holes. Three rubber washers cut from heavy rubber and the same size as the kodaloid washers are slid on the ends of the bolts down to the kodaloid washers.

The top of the chamber is then brought up on the opposite side and its three holes placed over the ends of the protruding bolts. A counter-pressure disc of Eastman heavy kodaloid —cut to fit the area of the chamber away from the main artery —is slid in underneath this piece and three nuts placed over the ends of the bolts and screwed down.

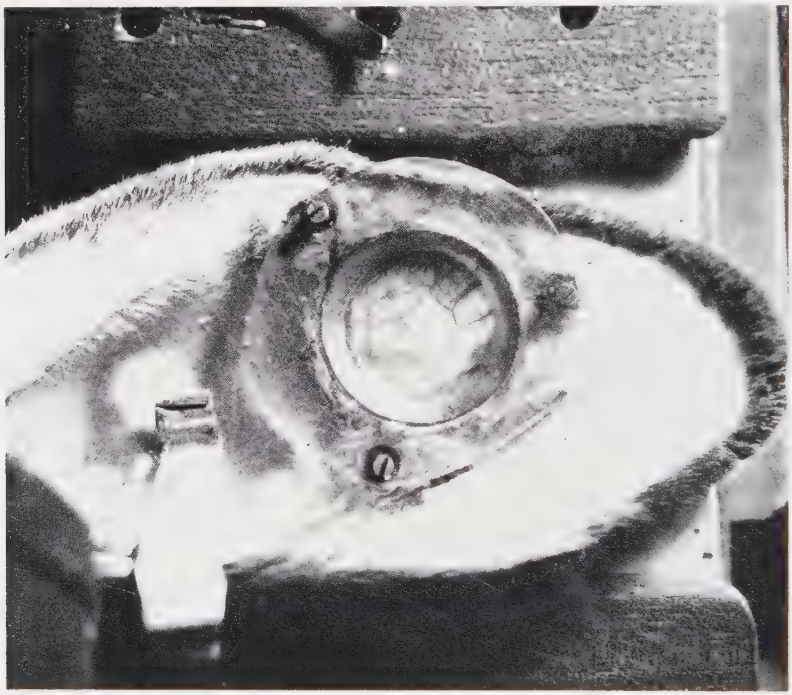

Fig. 6 Photograph of the ear of a rabbit in position for observation on the stage of the microscope, with a chamber of the 'preformed-tissue' type inserted in the ear. Natural size.

The rabbit is removed from the operating room and the chamber studied immediately under a binocular dissecting microscope. If the operation has been carried out with sufficient care, there should be comparatively little extravasated blood in the field of observation, and circulation in the large vessels should start a few minutes after the operation. The

pressure can be altered if necessary during the first half hour after operation by tightening or loosening the nuts. It is important to adjust them early to the optimum point—one in which as thin (and hence clear) an area of vessels as possible is obtained coincident with an active circulation. Figure 6 shows such a chamber in the ear. Figure 7 shows the vascular pattern obtained in the preformed-tissue chamber.

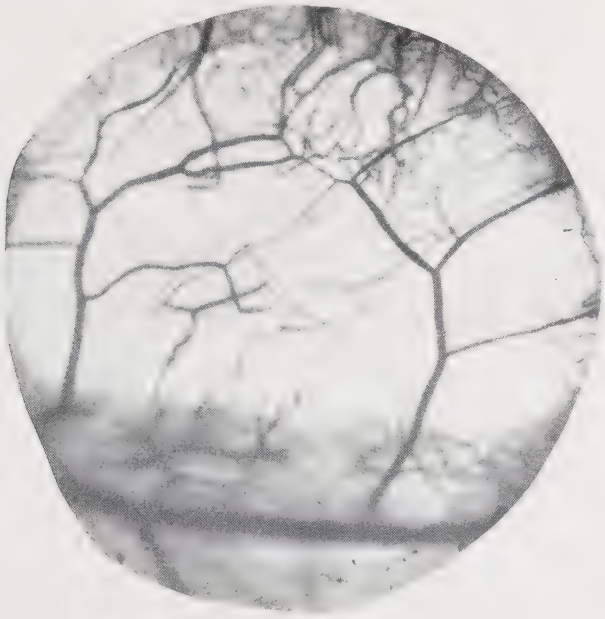

Fig. 7 Photomicrograph showing pattern of blood vessels in a 'preformed-tissue' chamber. The entire area of the chamber is shown. Photograph taken twenty days after insertion of the chamber. × 4.5.

Studies on the appearance, alteration, and reactions of the preformed blood vessels of these chambers have been carried out on a large series of rabbits, from the time immediately after the operation, over varying periods of a few weeks to several months in duration, with frequent photographic records. We have established the time at which such chambers recover from the initial disturbance due to the

operation and attain a stable condition in which accurate physiological observations and experiments can be carried out.

This type of chamber is studied chiefly with high powers of the dissecting binocular microscope and a bright light (point of light or a Delineascope) filtered through copper-sulphate solution or blue heat-resisting glass. It is satisfactory for studies of circulation and contraction of arterioles. Where finer studies of vessel walls are desired, the compound microscope is necessary. Albino or light-haired rabbits are preferable for this type of chamber.

For the study of the walls of capillaries, beautiful pictures may be obtained by removing the skin from the outer side under the binocular dissecting microscope, leaving an area of vessels and surrounding connective tissue behind. This procedure is involved in the 'combination chamber,' a description of which follows.

THE COMBINATION CHAMBER

This type of chamber was designed to make possible a comparison, in the same specimen, of living preformed blood vessels (present before the operation) with new-growing vessels. The insertion of the combination chamber is carried out in two stages. For the first stage, the construction of the chamber and the operative procedure are identical with those used in the case of the preformed chamber just described. A region, the center of which is as free as possible from large blood vessels, is chosen for the place of insertion at the time of the original operation.

At the time of construction of the first chamber, an extra bottom of the same size is cut out with a $^{13}/_{16}$-inch opening. Instead of gluing mica to this substitution bottom, a circle of Eastman heavy kodaloid, the same size as the viscoloid one, with a $^{1}/_{16}$-inch hole in its center, is glued to one side with ether-alcohol and the three screw holes burnt in the same positions as in the original bottom. A half-moon-shaped pressure disc of Eastman extra-heavy kodaloid is cut the

shape of the loose one made at the time of the original operation, and to it is glued a small clear round disc or table, made of two thicknesses of very clear Eastman heavy kodaloid. The whole is glued to this with ether-alcohol over the $\frac{1}{16}$ hole in the substitution bottom. As in the second type of chamber (the round table), especial care is taken to avoid the inclusion of air bubbles and dust in this central portion. To the top of this round disc the tiny kodaloid buffers described in the round-table chamber are glued with ether-alcohol.

Two or three days after the operation, circulation is in most cases well established in the chamber and much of the extravasated blood has cleared up. A photographic record of the vessels in the chamber is then made.

The second operation is carried out entirely under the binocular dissecting microscope. The glass stage of the microscope is washed with an antiseptic, and solutions and instruments sterilized as at the first operation. The ear is cocainized as before and the top of the chamber (with the mica cover) removed from the *outer* side, the skin washed with phenol (1 per cent) or metaphen (1 to 2500) and rinsed with sterile Ringer's.

The skin on this side is then dissected away slowly and carefully with a sharp-pointed iridectomy knife, or Bard-Parker scalpel, and fine forceps. Small twists of sterile gauze and sterile Ringer's solution are used to control the haemorrhage which invariably occurs. Care is taken to remove the hair roots which are often deeply embedded in the tissue.

A hole is then cut clear through the tissue in the center of the area, of the correct size and in the proper position for the insertion of the small table on the substitution bottom, which is then fitted into place. The ear is turned over and the nuts screwed down until the mica top is in contact with the buffers on the central table.

Thus, in the combination chamber there is an area in the center for the observation of new-growing vessels and a

peripheral area in which the preformed vessels are preserved (fig. 8), the new growth being in the center. In specimens in which the skin has been removed in this manner the walls of the preformed capillaries stand out with great clearness.

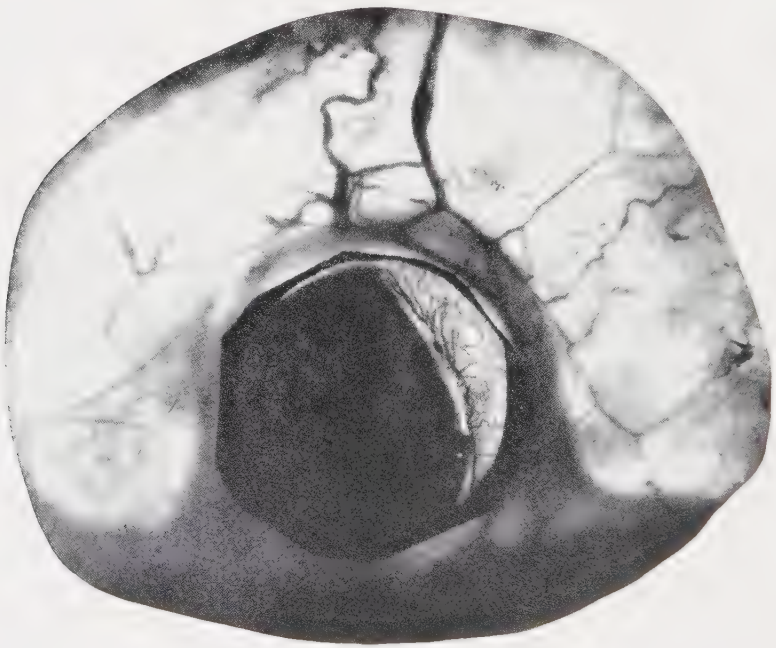

Fig. 8 Photomicrograph of a 'combination chamber' showing both preformed and new-growing blood vessels in the same ear. The outer circle shows the inserted disc with an area of new-growing vessels which have advanced partway across it. The black area in the center is caused by a piece of paper laid over the portion of the central disc on which the new vessels were not yet present, to exclude the excess of light. Photograph taken seventeen days after insertion of the original 'preformed-tissue' chamber and fifteen days after the reoperation and insertion of the central disc. × 6.

In fact, the pictures obtained in this type of chamber are in many respects the most excellent obtained in any. Moreover, the growth of new vessels onto the central disc takes place in four to five days—two to three days earlier than in the other new-growth chambers. However, the second stage of the operation is extremely slow and tedious. In the combination

chambers the epidermis regenerates very rapidly, evidently from small islands left at the time of the second operation, and soon invades the area of the central disc and blocks off the line of advancing blood capillaries. Beautiful pictures of epidermal growth are obtained in this way as well as interesting modifications in the mode of growth of blood capillaries, but the area of new growth after the first week or ten days is not so well adapted to long-continued high-power studies of individual vessels and cells as in the first two types described. At present, therefore, the combination chamber is reserved for the use for which it was designed, i.e., for short-time studies (over a period of a week to ten days) in which comparisons of the structure and contractility of preformed blood vessels with those of newly formed and new-growing vessels can be made in the same chamber.

The details of growth obtained in the studies of blood vessels and other cells which have been carried out with these various types of chambers will be described separately.

SUMMARY

The method of introducing transparent chambers in the rabbit's ear has now reached a stage in which an approximately standardized type of chamber is available for most types of investigation in which the use of the method is desirable.

The 'bay' chamber, in the form described here, is best adapted for long-continued observations of new-growing capillaries and of blood cells in a region undisturbed by motion, pulling and tension of tissue, and, with slight modifications, for studies of the effect of chemical solution, gases, etc., on living cells and vessels.

The 'round-table' chamber is adapted for general studies of the new growth of blood vessels and lymphatics, the formation of arterioles and venules from capillaries, and for cytological studies of blood cells and cells of the connective tissue. It is also best adapted for transplantation of other tissues, the introduction of bacteria, of foreign substances of a solid or semisolid nature, and for microdissection of tissues in vivo.

The 'preformed-tissue chamber' is best adapted for physiological and pharmacological studies on blood vessels in which it is important to be sure that vessels with intact nerve supply and normal surrounding tissue are present.

The 'combination' chamber is adapted for comparison, in the same specimen, of preformed vessels with new-growing and recently formed ones.

All of these methods are being used by a number of workers in this department and by several visitors from other departments in the investigation of a variety of problems. Some of the problems which are being studied at present are: growth of blood capillaries; growth of lymphatic capillaries; development of adventitial and smooth-muscle cells on the walls of blood vessels and relation of such cells to contractility; studies of blood vessels with the aid of moving pictures; appearance and behavior of blood and tissue cells; transplantation of bone, of bone marrow, of lymph glands, of kidney and of liver tissue to vascularized chambers; study of the growth of nerves and of the nerve supply of vessels; reaction of blood vessels to adrenalin, histamine, vasopressin, and other drugs; reaction of blood vessels in anaphylaxis; reaction of blood vessels, blood cells, and tissue cells to temperature and vital dyes and to various chemical solutions and foreign substances. With the working out of the normal growth, morphological, and reactive powers of living vessels and cells in the normal mammal—some of which has already been accomplished—the field of experimental physiological, chemical, and bacteriological experiments can be greatly widened.[3]

[3] The present writers have been engaged for the past year and a half in perfecting the technique of the various chambers just described. Other workers with the method and members of the staff have also cooperated with suggestions and also by trying out the various modifications in the course of their own investigations. Among these may be mentioned Dr. S. A. Matthews, who carried out a number of experiments on the permeability of the thin kodaloid to moisture and who aided materially in the development of the 'bay' chamber. The original suggestion of the use of the depressed bays was made by Dr. S. Culver Williams. The development of the glue which makes possible the construction of the chambers with mica covering is the work of Mr. B. B. Varian, who also has been of great assistance in the taking of photographic records of the chambers.

The work in this laboratory on the development of the method for studying cells and tissues in the transparent chambers inserted in the rabbit's ear is being aided by a five-year grant from the Rockefeller Foundation.

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STUDIES ON THE BLOOD OF THE FETAL ALBINO RAT

I. TOTAL COUNTS OF THE RED AND WHITE BLOOD CORPUSCLES

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TWO FIGURES

Preliminary to studies on the blood of the fetal rat under experimental conditions, a series of total counts of the red and white blood corpuscles of the normal fetus has been made. The only other blood counts of the fetal albino rat have been made by Addison and Richter (Donaldson, '24). Since only thirty counts were made, they were considered insufficient for comparative experimental purposes.

The youngest fetus from which we could obtain sufficient blood for counting with a standard hemocytometer was 15 mm. long (crown-rump length), or sixteen days old (Stotsenburg, '15). Moreover, in the youngest stages only enough blood could be obtained from one fetus for one count and a thin smear. Consequently, we took blood from one fetus for a white count and blood from another of the same litter for a red. Maternal counts of all litters were made and will be published later in a study of the relationship between pregnancy and blood-cell counts. In addition to these counts, we made some from postnatal rats at representative stages from the first postnatal day to the middle of the fourth postnatal month. Because of lack of data concerning the time of insemination and fertilization, we have arranged our results according to the crown-rump length of the rat fetus as expressed in millimeters.

METHODS OF STUDY

The procedure employed in making the counts was as follows: Laparotomy was performed on the unconscious mother by a single cut with a pair of scissors. The uterus was then opened over one fetus and the fetus shelled out with the amniotic sac intact. Upon removal of the placenta, there was slight hemorrhage, never exceeding a drop of blood. The amnion was then opened, the free fetus laid on its side, and its crown-rump length measured with a pair of fine calipers.

To draw the blood, the fetus was held in the right hand of the operator with its head down. The skin of the neck was slit with fine scissors, the diluting pipette held in readiness for immediate action, since the loss of even a drop of blood might ruin the sample, especially in the young fetuses. The jugular vein was then opened and the blood sample drawn and diluted. In order that the samples be fresh and from normal living fetuses, it was necessary to have a device for shaking the diluting pipettes in a uniform manner and at a constant speed. The agitator used was an electric motor geared to an eccentric, to which was attached a rod with clips between which the pipettes were inserted. The success of this device was evidenced by the uniform distribution of the cells within the counting chamber. The mixture obtained by use of the agitator was checked with mixtures obtained by manual shaking, and no apparent difference could be detected. The use of the agitator enabled us to use five fetuses from each litter and to have all the samples drawn and placed in the agitator within ten minutes after the uterus had been opened.

After procuring the samples for counting, a smear was made for future staining, in order that differential counts might later be made from this series of fetuses.

Hayem's solution and cresylecht violet (1:10,000) in 1 per cent acetic acid were used as diluents in making the red and white counts, respectively. The American standard haemocytometer with the double Levy counting chamber was used. In making the counts of the red corpuscles, the number in

eighty small squares was counted and four ciphers added to this number (factors of dilution × depth) to give the number of red blood corpuscles per cubic millimeter. In recording the white cell count, the cells in 6 sq.mm. were counted, three from each chamber. This sum was divided by 6 and multiplied by 200 (factors for depth × dilution). The nucleated red corpuscles were counted in a manner similar to the whites. An automatic tally counter was used to record the number of cells.

The cresylecht-violet solution was used in counting the white cells, because it was found that in unstained preparations it was difficult to discriminate between the white cells and the nucleated reds.[1] By the use of this stain, however, the nucleated red cells could be readily differentiated from the leucocytes by their small pycnotic nuclei and large clear cytoplasm. In the youngest stages the larger hemoblasts were counted as leucocytes, because they could not be discriminated from large lymphocytes. The difficulty with this method of counting the leucocytes in fetuses is discussed below.

Finally, measurements of the red blood corpuscles were made to check the size of the corpuscles as compared with the counts. The corpuscles measured were from smears stained with Wright's blood stain. Only smears with the same uniformity of preparation were selected. An ocular micrometer was used and the diameters of fifty cells from each smear measured. All measurements were made using an oil-immersion lens. Estimating the sizes of the corpuscles from these measurements, 1000 corpuscles were classified arbitrarily into three groups: 4 to 6 μ, 6 to 8 μ, and above 8 μ in diameter, respectively.

[1] We wish to acknowledge our indebtedness to Dr. E. W. Bray for suggesting the use of this solution.

OBSERVATIONS

The results of the counts of the red and white blood corpuscles and nucleated red corpuscles in this series of fetal albino rats are given in table 1 and in figure 1.

TABLE 1

Showing total counts of red and white blood corpuscles and nucleated red blood corpuscles per cubic millimeter of blood of fetal albino rats. The series is arranged according to the average crown-rump length of the fetuses in millimeters

LITTER NO.	NUMBER OF FETUSES	AVERAGE CROWN-RUMP LENGTH IN MILLIMETERS	AVERAGE TOTAL RED BLOOD CORPUSCLES PER CUBIC MILLIMETER	AVERAGE TOTAL WHITE BLOOD CORPUSCLES PER CUBIC MILLIMETER	AVERAGE TOTAL NUCLEATED RED BLOOD CORPUSCLES PER CUBIC MILLIMETER
H-8	5	15.4	694,000		
46	4	16.0	727,500	580	91,800
42	5	17.6	1,224,000	1963	160,315
H-7	5	18.4	1,118,000		
32	5	18.4	1,128,000	2180	
H-9	5	22.6	1,716,000		
34	5	23.0	1,470,000	4340	
33	5	23.0	1,780,000	3033	
H-5	5	23.5	1,788,000		
43	5	25.0	1,502,000	1680	41,900
18	5	27.4	2,142,000		
19	5	27.4	2,129,000		
H-6	5	28.4	1,870,000		
39	5	29.0	2,112,000	1633	22,250
H-17	5	29.1	1,929,000		
H-12	5	29.8	2,095,000		
H-14	5	29.8	2,176,000		
44	5	31.6	1,752,000		
H-16	5	33.4	2,078,000	1544	14,800
H-2 H-13	10	33.6	2,168,000		
35	5	33.6	2,006,000	2056	14,200
H-10	5	34.0	2,065,000		
38	5	34.2	2,144,000	2204	14,640
H-3	5	34.2	2,380,000		
H-15	5	34.4	2,206,000		
36	5	35.0	2,304,000	4272	
H-18	5	35.6	2,316,000		
15	5	37.2	2,458,000		
16	5	37.6	2,434,000		
17	5	38.0	2,996,000		
H-4	5	43.0	2.594,000		

Total litters studied, 32. Total number of fetal counts: red blood corpuscles, 159; white blood corpuscles, 55; nucleated red blood corpuscles, 35.

Examination of the counts of the red blood corpuscles given in table 1 and in figure 1 reveals the fact that, although there is a steady increase in the number of red blood corpuscles per cubic millimeter from the 15.4-mm. stage until birth (43 mm.), there are minor fluctuations. In none of these fluctuations does the count drop to as low as the earliest count. The most significant decrease is between the 31- and 32-mm. stages, or at about the middle of the period under observa-

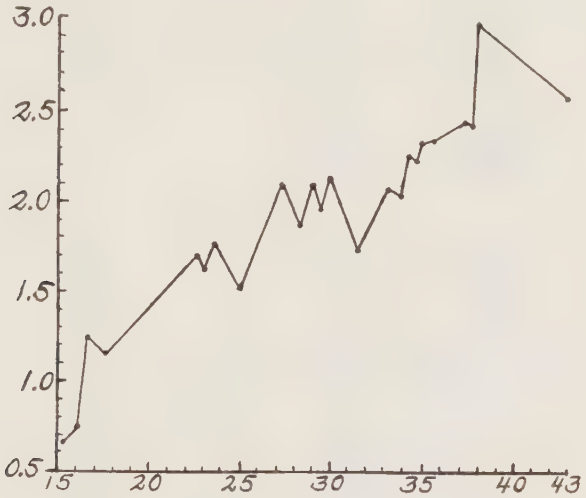

Fig. 1 Graph showing trend of count of red blood corpuscles per cubic millimeter in rat fetuses from data given in table 1. The abscissae indicate the length of fetuses in millimeters. The ordinates indicate the number of red blood corpuscles in millions per cubic millimeter.

tion. Such a decrease, preceded as it is by a fluctuation, may be interpreted as a major rhythm. A possible explanation for such a decrease might be a greater increase of the plasma at this time, thereby giving a greater dilution to the blood and consequently producing a decrease in the number of corpuscles per cubic millimeter. Another explanation might be the maturation of the large nucleated elements. The latter explanation finds support from the data in table 1 which show that there has been a definite decrease in the number of nucleated elements, culminating at the 31-mm. stage.

The decrease in the number of red blood corpuscles at the 32-mm. stage is followed immediately by a rapid rise, which continues until birth and reaches a level not hitherto encountered. Examination of Stotsenburg's figures for growth in

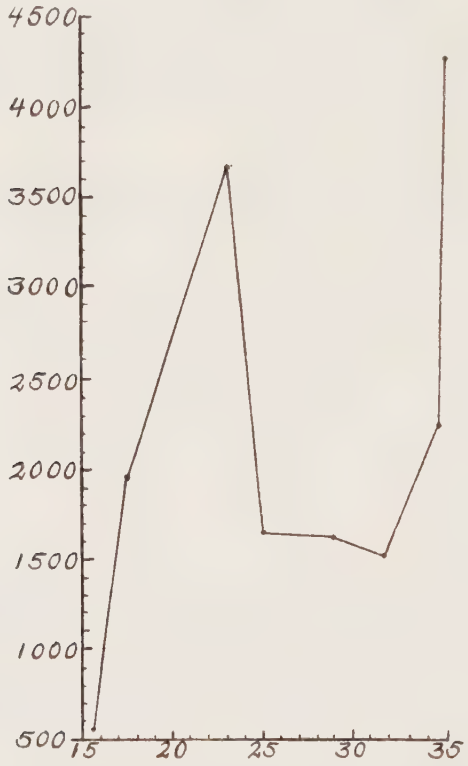

Fig. 2 Graph showing trend of count of white blood corpuscles per cubic millimeter in rat fetuses from data given in table 1. The abscissae indicate the length of fetuses in millimeters. The ordinates indicate the number of white blood corpuscles in thousands per cubic millimeter.

weight at this time shows that this is the period of most rapid growth in weight and length. Hence we may conclude that the increase in the number of red blood corpuscles is part of the general growth of the body as a whole.

Immediately upon birth, there is a decrease in the number of red blood corpuscles. The count is lowest three days after

parturition. The factor which suggests itself at once as accounting for this drop is the loss of the placenta together with the blood corpuscles which are caught in its vascular plexus. Hemorrhage from the newborn rat via the open umbilical vessels would also account for some decrease in the blood count. Another factor which may be concerned is the increase in the percentage of larger cells (table 3).

TABLE 2

Showing total counts of red and white blood corpuscles and nucleated red blood corpuscles per cubic millimeter of blood of young postnatal albino rats. The series is arranged in chronological order

RAT NO.	AGE	SEX	TOTAL RED BLOOD CORPUSCLES PER CUBIC MILLIMETER	TOTAL WHITE BLOOD CORPUSCLES PER CUBIC MILLIMETER	TOTAL NUCLEATED RED BLOOD CORPUSCLES PER CUBIC MILLIMETER
45-1	1 day	Female	2,480,000	2,000	2,000
45-2	2 days	Female	2,140,000	1,800	3,200
45-3	3 days	Male	2,240,000	2,240	3,000
46-1	3 days	Male	1,930,000	2,580	1,600
46-2	3 days	Female	1,950,000	3,040	1,200
46-3	3 days	Female	1,860,000	2,200	800
46-4	3 days	Female	1,860,000	3,000	1,800
8-1	4 days	Male	2,250,000		
8-2	4 days	Male	2,610,000		
8-3	4 days	Female	2,770,000		
8-4	4 days	Female	2,750,000		
8-5	4 days	Female	2,650,000		
8-6	4 days	Female	2,660,000		
3	5 days	Male	3,176,000		
11-1	5 days	Female	3,490,000		
11-2	5 days	Female	3,020,000		
11-3	5 days	Female	3,180,000		
7-1	1 week	Male	3,339,000		
7-2	1 week	Female	3,290,000		
7-4	1 week	Female	3,060,000		
24	2 weeks	Female	6,330,000	6,400	
23	6 weeks	Female	7,510,000	7,200	
25	7 weeks	Female	8,680,000	7,360	
29	3 months	Male	9,970,000	5,400	
27	3.5 months	Female	7,720,000	10,400	
28	3.5 months	Male	9,650,000	15,000	
30	3.5 months	Male	10,030,000	12,000	
31	3.5 months	Male	11,960,000	15,200	

By the end of the first week of postnatal life, the number of red blood corpuscles has increased greatly and reached a level never found in the fetus (table 2). From this time on there is a steady increase in the number of red blood corpuscles, so that by the middle of the fourth month the number characteristic of the adult is present.

The percentage increase in the number of red blood corpuscles per cubic millimeter from our youngest stage (15.4 mm.) to birth is about 500. During this interval the body as a whole has increased in length by about 250 per cent, whereas the weight has increased by nearly 900 per cent, or from about 0.525 gram to 4.630 grams (Stotsenburg, '15).

The leucocyte count as given in table 1 and figure 2 shows two distinct increases at 23 mm. and 35 mm., respectively. Starting with a relatively small count of 580 cells per cubic millimeter at the 16-mm. stage, the leucocytes rapidly increase in number, so that by the 23-mm. stage there are 3686 cells per cubic millimeter.

In the description of the procedure used in counting the leucocytes we have pointed out our attempt to discriminate between leucocytes and nucleated red blood corpuscles by using cresylecht violet in the diluting fluid. This stain enabled us to differentiate between those cells of the red series which had definitely become either erythroblasts or normoblasts, but the myeloblasts which are mother cells of both the red and white cells have been counted as leucocytes. Study of smears of the blood corpuscles of these early stages immediately makes clear the high leucocyte counts for this period. Consequently, after making a differential study of these smears and comparing the number of the myeloblasts with the numbers of definitely differentiated leucocytes, such as lymphocytes, premyelocytes, and mature granulocytes, we conclude that this first increase in the leucocytes is not actually a leucocyte increase, but a generalized hemoblastic increase including the mother cells of both the white and red blood corpuscles. The high count reached at the 23-mm. stage drops almost immediately, and accompanying it in the

differential counts is a marked decrease in the number of indeterminate hemoblasts.

The second increase in the leucocyte count is one in which only the true leucocytes are concerned, since differential counts of the blood smears of stages from 24 mm. on show a very definite increase in the number of neutrophilic granulocytes and practically an absence of hemoblasts. This decided increase in the leucocyte count coincides with the time just before birth when there is the greatest increase in the size of the body during the whole fetal period. Again, as in the description of the red blood corpuscles, we wish to call attention to the fact that this increase in the number of leucocytes is an expression of the general increase in the size of all of the organ systems.

The percentage increase in the number of leucocytes from the beginning of the period under observation until birth is about 700. At birth there is a decrease from nearly 3600 leucocytes per cubic millimeter to 2000, but this decrease is immediately followed by an increase (table 2). The decrease in the number of leucocytes at birth may possibly be explained by assuming that a number of cells are caught in the vascular channels of the placenta and are lost to the intrafetal vasa when the placenta is cut off. The increase in the number of leucocytes beginning with the third day may possibly be explained by regarding the increase as an expression of the response of the hemopoietic centers to the need for leucocytes to maintain an aseptic barrier in the region of the cut umbilicus. The number of leucocytes increases slowly during the first week, but by the middle of the fourth month the number of leucocytes characteristic of the adult rat is reached.

Although only thirty-five counts were made of the nucleated red corpuscles, we felt that since these counts were made at different stages they should be included. The nucleated red corpuscles, including megaloblasts, erythroblasts, and normoblasts, are most numerous at the beginning of the period under observation. From the 16-mm. stage to the 18-mm. stage these cells increase from 91,800 to 160,315 (table 1).

Following this high incidence, the nucleated red corpuscles gradually decrease in number, but they are still present in postnatal rats of the first week (table 2).

In table 3 are shown the percentages of three groups of red blood corpuscles as they occur in the measurements of the diameters of 1000 red blood corpuscles in smears. Unfortunately, we made no measurements of the living cells at the

TABLE 3

Showing the percentages of red blood corpuscles of three different sizes per 1000 cells in rat fetuses of the same series from which the total counts were made. Cell diameters are given in micra. Measurements are all made from blood smears stained with Wright's blood stain. This table should be compared with table 1

LITTER NO.	NUMBER OF SMEARS	AVERAGE CROWN-RUMP LENGTH IN MILLIMETERS	RED BLOOD CORPUSCLES, 4 TO 6 μ	RED BLOOD CORPUSCLES, 6 TO 8 μ	RED BLOOD CORPUSCLES, 8 μ AND ABOVE
H-8	1	15.4	56.9	27.7	15.4
46	2	16.0	22.5	33.1	44.4
42	2	17.6	19.6	37.1	43.3
H-7	1	18.4	25.4	25.9	48.7
H-9	1	22.6	13.8	23.4	62.8
34	2	23.0	1.4	73.6	25.0
33	2	23.0	0.8	81.1	18.1
43	2	25.0	1.0	84.7	14.3
18	2	27.4	2.2	88.3	9.5
19	1	27.4	1.8	84.8	13.4
H-6	2	28.4	7.8	81.6	10.6
39	2	29.0	5.0	84.8	10.2
H-12	1	29.8	1.8	90.2	8.0
H-14	2	29.8	1.6	89.6	8.8
44	2	31.6	1.3	87.4	11.3
H-16	2	33.4	3.7	85.4	10.9
H-2	2	33.6	4.4	83.6	12.0
H-13	2	33.6	4.5	89.2	6.3
35	2	33.6	2.7	83.8	13.5
H-10	2	34.0	1.6	92.0	6.4
38	2	34.2	1.2	88.5	10.3
H-3	2	34.2	5.4	89.6	5.0
H-15	2	34.4	1.7	87.7	10.6
36	2	35.0	1.3	84.9	13.8
15	2	37.2	1.0	90.2	8.8
16	2	37.6	0.6	91.6	7.8
H-4	5	43.0	0.4	84.2	15.4

time this study was made, so that attempts to estimate blood-cell volume of the fetus as compared with blood-cell volume of the adult were not carried out. However, since Knoll ('27) has estimated the blood cells per cubic millimeter in the human fetus on the basis of volume rather than by hemocytometric methods, we felt that measurements of the diameters of the cells at different stages might reveal some relation between size and count. When the cells were studied in the counting chamber, we noted that there was a relative scarcity of cells in the younger stages. Such small numbers of cells could not be attributed to size alone, but indicated an actual

TABLE 4

Showing the percentages of red blood corpuscles of three different sizes per 1000 cells in newborn rats. Cell diameters are measured. Measurements are all made from blood smears stained with Wright's blood stain

RAT NO.	AGE	4 TO 6 μ	6 TO 8 μ	8 μ AND ABOVE
45-1	1 day	0.7	82.1	17.2
45-2	2 days	0.9	80.0	19.1
45-3	3 days	1.4	83.2	15.4
47-1	3 days	0.5	87.5	12.0
47-2	3 days	0.7	81.2	18.1
7-1	1 week	0.1	91.5	8.4
7-2	1 week	0.6	85.6	13.8
7-3	1 week	0.8	94.7	5.5

low number of cells per cubic millimeter. The measurements made tend to support this view, since in the earliest groups there is a greater number of small cells than large or intermediate. These small cells are very minute and some of them measure less than 4 μ in diameter. Most of them are non-nucleated biconcave discs, others are micronormoblasts with a polychromatic staining reaction. Thus in the group that has the least number of red blood corpuscles per cubic millimeter the percentage of the smallest group of cells is highest. Almost immediately, however, the large cells increase in number, and there is a rapid rise in their number until the 22-mm. stage. Most of these cells are nucleated normoblasts. From these conditions it would appear that there is a great

production of normoblasts between the 16-mm. and 22-mm. stages. Following this stage the cells of the intermediate group increase in number and continue to be the most numerous by far of all of the red blood corpuscles. Reference to figure 3 and table 1 shows that at the 22-to-25-mm. stage there is a fluctuation in the count of the red blood corpuscles. In comparing this fluctuation with the percentages of the several types of red blood corpuscles it will be seen that the fluctuation occurs at about the time that there is a change in the type of dominant cell. It is possible that the fluctuation is an expression of the changes going on in the hemopoietic organs which lead to a greater production of corpuscles and a consequent increase in the total count together with an increase in the number of non-nucleated medium-sized cells. Except for minor fluctuations, the percentages of the three types of cells continue at about the same level from the 25-mm. stage until birth. At birth there is a slight increase in the number of large cells. This increase in the number of large cells continues for the first three days after birth (table 4). At the same time the total red-cell count decreases slightly (table 2). The decrease in the total count may therefore be caused by an increase in the percentage of the large cells. After the third day, the red-blood-corpuscle count gradually rises, while the size of the cells diminishes. In the adult rat the red blood corpuscles are all of the intermediate group, but overlap the lower group, the average diameter being 6.66 μ.

DISCUSSION

The counts of the red blood corpuscles given in this paper, although agreeing in general, within the error of the technique used, with the counts of the red blood corpuscles of the fetal rat as given by Addison and Richter (Donaldson, '24), show a lower count for the fetuses of the eighteenth day and higher counts for the succeeding days. It is probable that the discrepancy inheres in the difference in the number of counts made.

The counts of the leucocytes differ considerably from those given by Addison and Richter, notably as regards counts of the eighteenth day. The counts given here are much higher than those of Addison and Richter.

The counts of the nucleated red cells agree in general with those of Addison and Richter.

The average size of the red blood corpuscles at the seventeenth day of gestation is, in the fetal rat, 9.7 μ, according to Jolly (Donaldson, '24). In our seventeen-day group (16 to 17 mm.) the majority of the red blood corpuscles are 6 μ or above in diameter, and would probably average around the size given by Jolly. But it must be pointed out that 20 per cent of the red blood corpuscles at this time have a diameter below 6 μ. Beyond this stage there is a gradual decrease in the diameter of the cells.

The smallest red blood corpuscles, which predominate at the 15.4-mm. stage (sixteenth day), suddenly show a sharp numerical decrease at the 23-mm. stage, and from that time on are present in very low percentages. The drop in the percentages of the cells follows immediately upon a tremendous rise in the number of the large cells, most of which are normoblasts. Going back to stages in embryonic life earlier than any which we have included in our total blood counts, we find that at the 10-mm. stage, or during the fourteenth day, blood smears reveal only the presence of nucleated ancestral hemoblasts. Since the small red blood corpuscles are not present at this time and are present in maximum numbers at the 15.4-mm. stage (sixteenth day) and practically disappear on the nineteenth day (23 mm.), we conclude that the life tenure of the small red blood corpuscle is between four and five days. Small numbers are added from time to time, but the bulk of these smallest corpuscles are eliminated from the blood during the nineteenth day. The fluctuation of the total count at this time (fig. 1) may be an expression of this change. The life tenure of the other types of cells cannot be estimated from our figures.

Comparison of our data for the rat fetus with those of Knoll ('27) for the human embryo and fetus shows marked differences. Knoll estimated the number of cells that should be present per cubic millimeter of whole blood by measuring the red blood corpuscle in the living condition and comparing the average volume of the cells of the fetus with that of the adult. Knoll's method assumes that the cell-plasma ratio is the same in both fetus and adult. Reckoning on this basis, Knoll gives a count of 3,500,000 red blood corpuscles per cubic millimeter for fetuses 170 mm. long, or about five and one-half months old. No nucleated red blood cells were found in the blood of the human fetus after the sixth month. In the rat the level of the fetal red-blood-corpuscle count never reaches as high when compared with the adult count as does that in the human fetus. The highest count for the fetal rat, i.e., 2,996,000, is less than 30 per cent of the adult count, and this occurs only during the last day of gestation. In the human fetus 30 per cent of the adult count is reached during the third month, or approximately at the end of the first third of gestation. Furthermore, nucleated red blood corpuscles occur in numbers sufficient to be counted in the rat for the first week after it is born.

From the study of our material we conclude that the increase in number of red blood corpuscles during the latter part of gestation in the fetal rat signifies essentially an increase by addition of new cells of approximately the same size as those already present. In other words, our evidence indicates that the cell-plasma ratio is less in the fetus than in the adult. Examination of a blood sample in the counting chamber reveals an actual smaller number of cells in the field than is the case for the adult, and they are not as closely crowded as in a sample of adult blood. If the difference in the number of cells between fetus and adult were only a question of the difference in size of the cells, we should expect the cells to be as closely crowded in one case as in the other. We grant, however, that part of the difference in the respective counts may be due to size, but we are not ready to

admit that this is the only factor which is to be taken into consideration in making a blood count.

In contrast with the count of the red blood corpuscles of the human fetus as reported by Knoll is the leucocyte count as estimated by the same investigator. Again, the indirect method was used in estimating the number of leucocytes per cubic millimeter. Starting with a relatively low count of 1120 leucocytes per cubic millimeter in a 12-mm. embryo, the leucocyte count rises to as high as 28,000 at the 160-mm. stage. Assuming the normal leucocyte count of the adult to be 8000 to 12,000 cells per cubic millimeter, the number thus given for the fetus of the fifth month is more than twice as much. In the rat fetus the leucocyte count, although it shows great fluctuation between the 18.4-mm. stage (eighteenth day) and the 35-mm. stage (twenty-first day), never reaches a level half as high as the count of the adult rat. The explanation for the differences in the leucocyte counts of the fetal rat and man may lie in the relatively late development of the leucocytopoietic centers in the rat.

SUMMARY

1. The total count of the red blood corpuscles per cubic millimeter of blood is given for a series of rat fetuses from 15.4 mm. crown-rump length to 43 mm. crown-rump length, or from the sixteenth to the twenty-second day of gestation.

2. Total counts are given of the leucocytes and nucleated red cells of representative stages from this same series of fetuses.

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IMPROVED METHOD OF REFRIGERATION OF WHOLE BLOOD FOR CENTRIFUGATION

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ONE CHART AND THREE FIGURES

FOREWORD

Every one who has centrifugated whole blood to obtain the supernatant plasma usually employed as a medium for the in-vitro growth of living tissue is familiar with the absolute need of thorough refrigeration of the blood to obviate premature coagulation.

In the method universally employed by tissue culturists, a degree of refrigeration is obtained by the use of powdered ice placed around the paraffined centrifuge tubes in which the blood is subsequently collected. The centrifuge tube with its contained blood is then transferred to a trunnion-cup, and the space between the tube and the inner surface of its container is packed with powdered ice preparatory to centrifugation. In laboratories where the whole blood is centrifugated twice, first at low speed and then at high to obtain an absolutely cell-free plasma, the centrifuge tube is again surrounded by powdered ice before its final centrifugation. It will be conceded by those who have performed the various operations of powdering and packing the ice, briefly detailed above, that the work is time-consuming, and at best the subsequent refrigeration is rather unsatisfactory, principally because of the impossibility of maintaining a sufficiently constant degree of frigidity around the centrifuge tube while in operation.

The gradual loss of frigidity in the trunnion-cup while rotating may be attributed chiefly to the following physical factors: 1) to the generation of heat induced by the air resistance encountered by the trunnion-cups during their rapid centrifugal flight; 2) to the transmission of this heat to the air between the particles of ice, causing their rapid melting; and, 3) to the rapid conduction (by the water resulting from the melting ice) of heat to the centrifuge tube containing the blood. The rise of temperature due to these three causes cannot be absolutely prevented by any experimental device. It may, however, be greatly retarded by a procedure to be described in the present paper. A comparison of the temperatures obtained after equal time intervals by the old and the new methods, respectively, will be found in the table (fig. A). To cite any one of the results there tabulated, it is found that at the end of a five-minute period of centrifugation the temperature obtained by the old method is 77°F., that obtained by the new method, about to be described, is 48°F. It is evident that a difference of 29°F. in the centrifugated blood must be of great importance in retarding the action of any factor that may influence premature coagulation of the plasma.

While the present method of refrigeration is extensively employed and yields passable returns, the innovation here suggested must have all the advantages that come from increased refrigeration. It also has, as will presently be seen, the additional advantage of greatly facilitating manipulation.

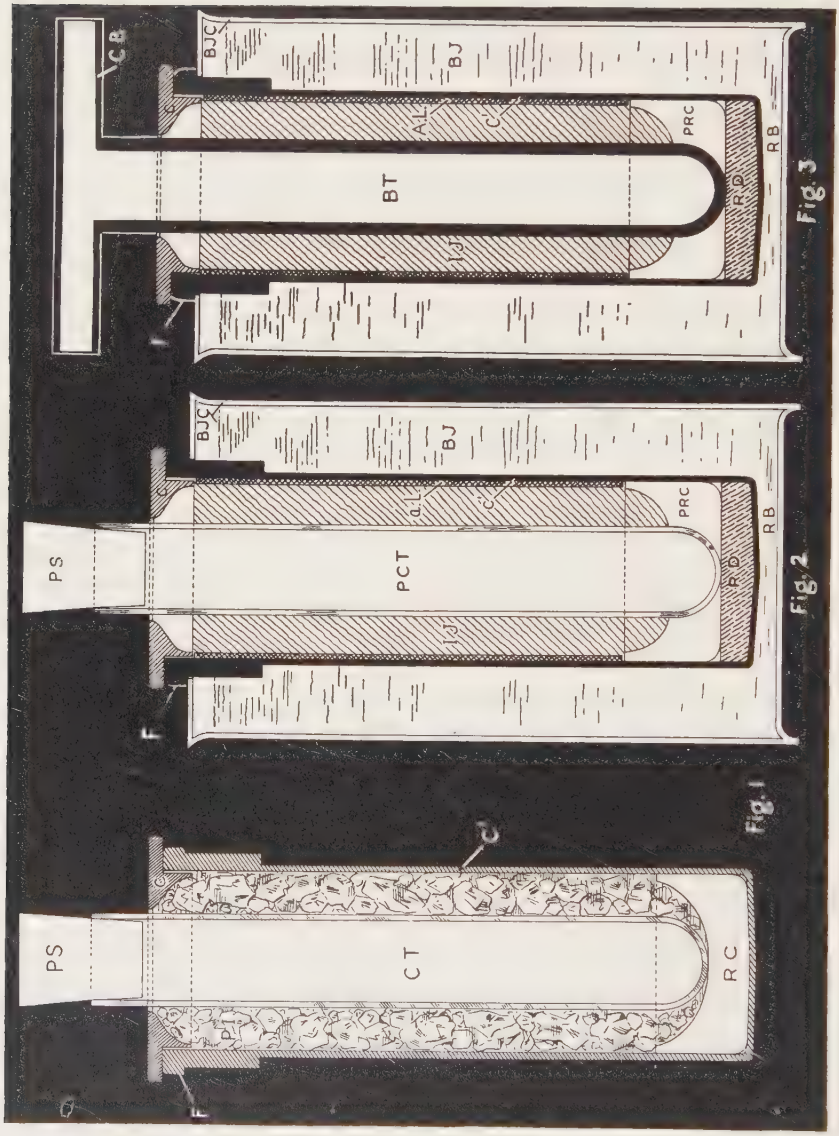
IMPROVED METHOD OF REFRIGERATION

With one exception, all of the apparatus employed in the method in use at the present time remains available for the new; the essential parts of the centrifuge, consisting of the trunnion-cup (fig. 1, C^1), rubber cushion (fig. 1, *R.C.*), and collar (fig. 1, *C.*) remain unchanged. The one exception affects the rubber cushion at the bottom of the trunnion-cup, which must be subjected to the following slight modification:

Removing the rubber cushion (fig. 1, *R.C.*) from the cup, this cushion is centrally perforated (fig. 2, *P.R.C.*), the diameter of the perforation being so chosen as to allow the snuggest possible union between the paraffined centrifuge tube (fig. 2, *P.C.T.*) and the inner wall of the cushion perforation (fig. 2). This arrangement answers a two-fold purpose: 1) It overcomes the tendency of the closed centrifuge tube to float upward (because of its lesser specific gravity) before the freezing of the water occurs to hold it in its proper position; and, 2) it brings the axis of the centrifuge tube into coincidence with the axis of the trunnion-cup, thus assuring a uniform thickness to the layer of ice which (as later described) is to fill the space between the centrifuge tube and the trunnion-cup.

One of the two slight additions to the usual centrifuge accessories enumerated above is the circular rubber disk illustrated in figure 2, *R.D.* The circumference of the disk is such as to allow it to fit readily into the trunnion-cup and it is approximately 3 mm. thick. Its function is to cushion the centrifuge tube during the process of centrifugation; hence it is placed on the bottom of the trunnion-cup immediately beneath the perforated cushion and the lowest point of the convex bottom of the centrifuge tube (fig. 2). The remaining addition to the centrifuge accessories is the asbestos insulation (figs. 2 and 3, *A.L.*), with which the trunnion-cup is lined. The flexible asbestos foil[1] (1 to 5 mm.) is rolled into cylindrical form, allowing a slight overlap, and inserted into the trunnion-cup. Its lower end rests on the peripheral edge of the perforated cushion, while the upper end terminates about 6 mm. below the upper edge of the trunnion-cup, in order to permit the proper seating of the collar *C.* (figs. 2 and 3). The asbestos cylinder is allowed to unroll within the trunnion-cup, the force of recoil insuring its close contact with the inner wall of the container. If in some instances this force proves insufficient, the asbestos lining can be adapted with the

[1] The asbestos lining used in the trunnion-cups will last indefinitely, if properly prepared and saturated with shellac.



Figures 1 to 3

fingers to the surface of the container. The asbestos cylinder is then partially withdrawn from the cup, and the exposed seam is glued or fastened by a metal staple.

It is now completely withdrawn, dipped two or three times in a good grade of orange shellac, and returned to the cup while still wet. The excess shellac gravitates to the bottom of the cup; rolling the cup between the fingers will distribute the shellac in an even layer over the perforated cushion and the rubber disk beneath. When thoroughly dry, the orange shellac forms a water-proof coating for the asbestos foil, as well as establishing a firm attachment between it and the inner surface of the trunnion-cup.

The various parts having been assembled as described, a properly sealed paraffined centrifuge tube is inserted in the circular aperture of the rubber cushion (fig. 2, *P.R.C.*). The space between the asbestos-lined inner wall of the cup and the paraffined tube (figs. 2 and 3, *I.J.*) is filled with water, which when frozen forms an ice-jacket surrounding the tube. The collar (fig. 1, *C.*) is then placed in position; the ensemble is immersed in a brine-jacket[2] illustrated in figures 2 and 3, *B.J.* The brine-jacket is formed of a piece of brass tubing (outside dimension, 63 × 100 mm.) with a slightly raised base (fig. 2, *R.B.*) to prevent it from adhering, while in the

Fig. 1 Represents the unit now in use for centrifugating whole blood or other material. *C*[1], trunnion-cup; *R.C.*, rubber cushion; *C.*, aluminum collar; *P.S.*, paraffined stopper; *P.I.*, powdered ice surrounding the paraffined centrifuge tube; *P.C.T.* and *F.*, the flange encircling the opening of the trunnion-cup.

Fig. 2 Improved ice unit. Abbreviations as before. In addition: *A.L.*, asbestos lining covering the inner wall of the trunnion-cup; *R.D.*, rubber disk to cushion the tube, *P.C.T.*, when *P.R.C.* is perforated; *I.J.*, ice-jacket when water solidifies; *B.J.*, brine-jacket; *B.J.C.*, brine-jacket cover; *R.B.*, raised bottom; *F.*, flange of trunnion-cup resting on the cover, *B.J.C.*

Fig. 3 Replacement unit. Abbreviations as before. In addition: *C.B.*, cross-bar, and *B.T.*, brass tube, whose removal leaves a cylindrical channel in the ice-jacket.

[2] An ordinary aluminum cup or water tumbler was the type of brine-jacket employed by the writers in their early experiments, with excellent results. In these experiments a hard paper collar coated with paraffin was used to centralize and support the trunnion-cup in its container.

freezing chamber, to the surface of the metallic shelf. The upper opening of the brine-jacket is closed by an annular metal collar (fig. 2, *B.J.C.*) attached to the vessel, with a central perforation whose diameter is sufficient to accommodate the passage of the trunnion-cup. The cup is allowed to descend until the lower edge of the elliptical flange (fig. 2, *F.*) surrounding its mouth rests on the annular collar. The height of the brine-jacket, as mentioned above, permits the trunnion-cup to hang suspended on its flange, thus allowing sufficient space between its bottom and the base of the brine-jacket for the circulation of the fluid surrounding it.

This construction of the unit is such as to eliminate any possibility of accidental displacement while being conveyed from place to place or in the handling of the ensemble during the process of bleeding. Having assembled the various parts of the device, the brine-jacket is filled with a saturated solution of sodium chloride and placed in the freezing chamber of an electric refrigerator.

During the process of blood collection, the ensemble (fig. 2) is removed from the refrigerator; the cork and upper end of the centrifuge tube protruding above the cover (fig. 2, *C.*) are swabbed with alcohol to remove the congealed moisture which accumulates on the exposed parts of the unit during the course of freezing. Active bacterial contamination is as obviously impossible in the new method as in the old; moreover, at the temperature conditions under which the device is used, whether in or out of the refrigerator, any organism contaminating it must be rendered innocuous for a considerable period of time through latency of development.

From this stage on, the process is similar in all respects to the method previously employed. Sufficient blood having been collected in the extremely cold centrifuge tube[3] (constantly encased in its thick and solid layer of ice), the tube

[3] The amount of collected blood may be ascertained by the use of a graduated pipette or any other similar means. The pipette is inserted into the centrifuge tube, the upper end is sealed by the index finger, and the pipette withdrawn. The column of blood contained in the tube indicates the number of cubic centimeters contained in the vessel.

is resealed, the trunnion-cup is removed from the icy brine, placed in the centrifuge, and its contents centrifugated.

REPLACEMENT UNIT

In laboratories where whole blood is centrifugated twice, a number of replacement units are prepared. Figure 3 illustrates this unit preparation, using a brass tube (fig. 3, *B.T.*) instead of the centrifuge tube (fig. 2, *P.C.T.*). The diameter of the brass tube is somewhat greater than that of the centrifuge tube ordinarily used. This difference in diameter is essential in order that the removal of the brass tube may leave a tubular channel larger in diameter than the replacement tube, permitting the latter to be readily seated in its proper position. This would be quite impossible if the tubular channel were of equal diameter with the replacement tube; for under these conditions, any attempt to insert the latter would result in the formation of an air cushion beneath the tube, precluding any possibility of seating it properly.

A sufficient length of the brass tube is allowed to extend above the collar (fig. 3, *C.*), terminating in a cross-bar (fig. 3, *C.B.*), to facilitate its removal. When the brass tube has been removed, the unit presents a cylindrical or tubular channel of solid ice which freely admits the centrifuge tube containing the blood transferred from the unit previously used. The process of centrifugation can then be continued under unchanged conditions of intense refrigeration.

From the preceding description it should be evident that when once the slight but necessary changes are made in the centrifuge accessories, the maintenance of an ample supply of ice units for the refrigeration of whole blood becomes an extremely simple matter. It entails no additional effort other than the replacement of paraffined tubes and the refilling of trunnion-cups with water.

Comparison of results obtained by old and new methods, respectively

Experiments were made by which, under exactly analogous conditions of centrifugation, the rate of increase of temperature during regulated time intervals could be determined for the method now employed and the one here described.

In order to eliminate any possible difference of conditions in the experiments, two sets of paraffined centrifuge tubes were selected. The tubes of the first set were placed in a vessel containing powdered ice; those of the second were prepared according to the method just described. The two sets of tubes were then stored in the freezing chamber of the refrigerator and allowed to remain until the water surrounding the tubes in the new unit was solidly frozen. The two units, one a tube treated by the old method, the other a unit of the new, were then simultaneously withdrawn from the refrigerator, and into each centrifuge tube were pipetted 5 cc. of water at 98°F. Before inserting in the centrifuge, the trunnion-cup of the old-method unit was packed with powdered ice until its weight was brought into exact equivalence with that of the new unit.[4] The two units were then inserted in the centrifuge trunnion rings, at points diametrically opposite each other, and rotated (V. = 3800 R.P.M.) for a measured interval of time. Then, without removing the centrifuge tubes from their trunnion-cups, the respective temperatures of the contained fluids were read on separate thermometers.[5] This experiment was performed twice under the same condition of centrifuge room temperature (89°F.) for each time interval of a series of intervals ranging from

[4] It will be noted that, owing to the diminished capacity of the trunnion-cup, as a result of its additional cushion and asbestos lining, the actual weight of ice in the improved unit was found to be less than the weight of ice in the present unit by 10.2 grams.

[5] The two thermometers employed in obtaining the temperature readings throughout the experiments were carefully and repeatedly compared as to accuracy, both in room temperature and in the known temperature of the refrigerator.

one to eight minutes. The results are recorded in the following table.

Temperature of fluids prior to centrifugation 98° F. Velocity of centrifuge = 3800 R.P.M.

A	B	C	D	E
1′	2′-30″	52.0°	35.0°	17.0°
2′	2′-35″	64.5°	38.0°	26.5°
3′	2′-36″	66.0°	40.5°	25.5°
4′	2′-31″	70.0°	44.0°	26.0°
5′	2′-32″	77.0°	48.0°	29.0°
6′	2′-32″	81.0°	54.0°	27.0°
7′	2′-31″	84.0°	58.0°	26.0°
8′	2′-33″	87.0°	68.0°	19.0°

Fig. A Comparative temperature readings of centrifuge fluids, employing present and improved methods of refrigeration. A = Period of centrifugation. B = Time required for centrifuge to come to rest at conclusion of each experiment, after discontinuance of power. C = Temperature of fluid following each experiment under conditions governing the method of refrigeration now commonly used. D = Temperature of fluid following each experiment under conditions governing the improved method of refrigeration. E = Differences in temperature of the fluids following equal and simultaneous periods of centrifugation under the respective conditions governing the two methods of refrigeration.

CONCLUSIONS

1. The improved unit method insures a lower and more constant refrigeration.

2. The asbestos lining forms an insulating barrier, and to a great extent precludes the passage of the heat usually transmitted by the trunnion-cup to the interior.

3. The new method eliminates the necessity of powdering and packing ice around the paraffined centrifuge tube.

4. The brine-jacket maintains the frigidity of the trunnion-cup and its contents when removed from the refrigerator until such time as the cup is placed in the centrifuge.

5. The replacement unit employed for continued centrifugation is just as efficient as the primary unit, and the transfer of the centrifuge tube containing the blood can be accomplished in a fraction of the time required to repack the old unit with powdered ice.

6. Balancing of the improved units in preparation for centrifugation is unnecessary, as the possible variation in weight is negligible.

The authors desire to acknowledge their appreciation for the earnest cooperation accorded them by Miss Margaret K. Davis, their assistant.

THE BLOOD CYTOLOGY OF DOGS[1]

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SIX FIGURES

A search of the literature reveals but scanty information as to average or 'normal' values or standards for the dog under controlled conditions or for various lengths of time, although this animal has been very commonly used in the study of changes involving the hematopoietic system. The desirability of such data is obvious. The establishment of average 'normal' indices requires a large number of values necessarily obtained under varying conditions. The data here presented are still inadequate in this respect, but they are submitted in the hope that workers in other laboratories in which the dog is extensively used will be stimulated to add their findings, so that ultimately the number of values will justify the establishment of 'normal' levels.

This report includes 178 determinations on sixty 'normal' animals (thirty-seven males and twenty-three females), of various breeds and ages, obtained from the pound and used in various investigations. When received, each animal was bathed, purged, and placed in a large, roomy, outside cage and fed a standard diet for at least a week before any determinations were begun. At this time animals that refused to eat or appeared unwell were discarded. In sixteen experiments the Cowgill ('23) diet was fed; in the remainder, the bread S of Whipple and Robscheit-Robbins ('25) with salmon added as an appetizer. Both of these diets are tolerated by

[1] Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

dogs without loss of appetite for considerable periods. The indiscriminate use of pound animals for work of this sort is open to criticism, but by careful selection and allowance for proper control periods it is believed that truly representative results are obtained.

Routine examinations were usually made in the morning. Blood was taken from the ear, the first few drops being discarded. Larger quantities, when necessary, were drawn from the femoral vein or artery. Duplicate counts were always made at the same time of day by one person, using the same pipettes and apparatus throughout. Platelets were counted by the direct method of Rees and Ecker ('23). Hemoglobin was determined with the Newcomer hemoglobinometer, calibrated by oxygen-capacity determinations in the Van Slyke and Neill manometric apparatus. Reticulocytes were counted by the method of Haden ('23 a), using brilliant cresyl blue and counterstaining with Wright's. Exceptionally fine preparations are obtained by this method. Three hundred red cells were counted, the number of reticulocytes in corresponding fields noted, and the total number computed from the ratio. Cell volume was ascertained in Van Allen hematocrits, using 1.6 per cent sodium oxalate, which, as Whipple has pointed out, is isotonic with dog's blood, and centrifuging for half an hour at 3200 revolutions per minute; plasma volume, by the vital-red method; specific gravity, according to Barbour and Hamilton ('26); fragility, by Silvette's method ('28); differential counts, by Feemster's method ('28), which makes use of two solutions of Wright's stain in methyl alcohol and ethyl alcohol, respectively, and buffered water for rinsing and washing the slides.

Following the method of Ponder ('24), the 'corpuscular volume' or average volume of the individual red blood cell was obtained by dividing the volume of cells per 100 cc. (hematocrit) by the number of cells in the same volume (red-cell enumeration) and is expressed in cubic micra. The 'corpuscular Hb,' or amount of Hb per cell, was obtained by dividing the Hb per 100 cc. of blood by the number of cells, as in

the former case, and is expressed in grams $\times 10^{-12}$. This calculation is preferred to that of the color index, as representing a more absolute value, since the latter is calculated in terms of per cent Hb, which is a variable figure depending on the method and standard used. The 'per cent saturation,' or actual proportion of the substance of each cell taken up by Hb, was obtained by dividing the corpuscular Hb by the corpuscular volume, or directly by dividing the Hb content expressed in grams per 100 cc. by the hematocrit value, expressed in cubic centimeters per 100 cc.

At least two sets of determinations were made on alternate days on each animal. The observation periods vary from one to fifteen weeks. The actual number of determinations made on each dog is indicated in table 1, which gives the average for each animal and the mean of these averages. The experiments were distributed fairly evenly throughout the year. No dates are given, since an analysis of our results showed no significant seasonal variations. Our values are too few in number to obtain any correlation for sex or body weight.

The average of 101 determinations of specific gravity on thirty-four animals is 1.0503, with extremes of 1.0420 and 1.0562. Stebbins and Leake ('27), using the same method, found values of 1.0453 to 1.0644 for 11- to 18-kg. dogs. Many animals show little or no variation over periods of five and six days. Changes that do occur, on the other hand, are not easily correlated with the number of reds or the Hb content. The average red-cell count in the sixty animals is 6.16 million, with extremes of 4.86 and 8.56 million. Frequency-figure 1 shows the distribution in our sixty animals. From it we see that counts at any point from 5.2 to 7.6 million are common, 87 per cent of them falling within this range. The average is indicated in the chart (similarly in figs. 2, 3, 4, and 6) by a column having oblique lines in the lower portion. Our values are in fair agreement with those obtained by others. In forty-seven ordinary street dogs, judged from their general condition to be normal, Musser and Krumbhaar ('14)

TABLE 1

DOG NO.	SEX	WEIGHT, KG.	NUMBER OF DETERMINATIONS	SPECIFIC GRAVITY	R. B. C., MILLIONS	RETICULOCYTES, PER CENT OF R. B. C.	Hb, GRAMS PER CENT	CORPUSCULAR Hb, GRAMS × 10-12	HEMATOCRIT, PER CENT	CORPUSCULAR VOLUME, CU. μ	SATURATION, PER CENT	FRAGILITY HEMATOCRIT READINGS IN PER CENT Per cent $Na_2C_2O_4$ 0.5	0.6	0.7	0.8	PLATELETS × 1000	WHITES	DIFFERENTIAL S	L	N	E	B
1	♀	9.6	11		5.52		10.57	21.9	33.0	66.0	35.6					488	13,254	18	4	69	7	0
2	♀	7.4	8		6.10		12.88	21.2	39.7	65.7	32.5					500	15,822	17	4	71	6	0
3	♀	8.0	2	1.0492	5.53		11.39	20.0	30.0	54.3	38.0					650	9,250	18	7	75	0	0
4	♀	11.6	6		6.11		13.09	21.4	42.0	68.7	30.9					536	11,760	19	3	72	6	1
5	♀	8.2	4	1.0423	4.86		10.08	20.8	27.6	57.8	37.6					520	6,000	24	7	69	0	0
6	♀	11.8	3	1.0505	7.23		12.20	16.1	42.0	58.7	27.6					512	9,333	9	5	86	0	0
7	♀	16.6	8	1.0441	5.93		10.59	18.8	32.4	58.0	32.2					552	7,150	9	5	81	0	0
8	♀	14.5	3	1.0491	6.04		10.13	16.7	27.5	45.5	36.6					550	7,673	18	9	72	5	1
9	♀	11.4	15		5.54		12.87	23.3	39.0	70.4	33.3					500	13,513	18	3	75	5	0
10	♀	13.0	2	1.0460	5.25		11.79	22.4	32.0	60.9	36.8					480	19,200	18	2	75	5	0
11	♀	14.0	2	1.0470	6.83		13.00	19.0	42.0	61.5	30.9					550	13,200	24	1	75	3	0
12	♀	14.0	2	1.0420	5.22		11.59	22.1	35.0	67.1	32.9					554	13,200	20	1	75	4	0
13	♀	7.2	3	1.0503	6.88		13.10	19.0	41.3	60.1	31.6					532	11,666	19	2	75	4	0
14	♀	10.4	4	1.0550	6.81		13.28	22.7	40.5	69.9	32.5					500	16,700	20	2	75	2	0
15	♀	8.0	5	1.0430	5.05		10.71	21.1	26.0	41.4	41.2					504	11,572	19	2	77	2	0
16	♀	7.2	2	1.0450	5.40		10.67	19.6	32.0	57.7	43.2					524	13,980	17	2	75	6	0
17	♀	11.0	4	1.0520	5.98		10.36	17.3	46.2	79.2	21.9					400	13,600	18	10	76	1	0
18	♀	11.4	3	1.0505	5.99		11.17	18.8	48.3	81.3	23.0					430	16,666	18	7	75	0	0
19	♀	10.2	2	1.0528	7.16		10.82	15.0	49.0	74.5	20.2						17,350	23	5	72	0	0
20	♀	14.2	4	1.0474	6.54		10.27	15.6	45.2	69.4	26.8						12,688	18	6	80	1	0
21	♀	14.5	2	1.0545	6.36		13.90	16.6	42.5	50.8	32.6					370		24	5	69	2	0
22	♀	11.4	2	1.0560	7.44		15.20	20.4	45.5	61.1	33.3					520	12,600					
23	♀	10.8	2	1.0562	8.04		13.90	18.5	43.5	54.1	42.5					700		21	8	70	1	0
24	♀	16.0	2	1.0482	4.96	1.33	10.65	21.2	32.2	65.1	32.6					962	10,100	19	7	72	2	0
25	♀	12.4	2	1.0430	7.32		13.90	19.2	37.0	51.1	37.5					530	9,600	26	8	64	2	0
26	♀	21.0	2	1.0560	8.09	1.00	14.09	19.2	36.0	44.4	39.1					540	10,000					
27	♀	14.0	2		5.72		14.80	25.8	33.5	58.5	44.1											
28	♀	9.4	2		6.89	1.00	13.11	19.0	37.0	53.6	35.5	30	70	55	59	670	15,000	28	8	63	1	0
29	♀	10.0	2		7.43	1.66	14.91	20.1	39.5	53.0	37.5	31	73	75	63	1022	11,450	26	6	67	1	0
30	♀	13.4	2		8.56	1.33	15.59	18.7	43.0	50.2	37.4	5	40	38	37	630	10,726	21	5	74	0	0
31	♀	9.6	2		7.11	1.33	12.91	18.2	32.0	45.9	38.7	54	59	51	46	1275	16,625	22	4	73	2	1
32	♀	9.0	2		6.64	1.00	15.00	22.8	43.2	60.3	34.6	50	58	54	43	575	10,550	25	6	68	1	0
33	♀	8.4	2		6.46	1.00	13.32	20.6	38.2	59.2	34.9	60	63	61	55	465	14,375	27	4	73	0	1

34	♀	8.0	2		7.38	1.08	15.49	20.9	43.7	59.2	35.3	31	75	73	66	850	9,920	34	10	56	0	0
35	♂♀	8.2	2	1.0510	7.13	1.00	14.16	19.8	39.0	53.3	37.1	69	73	64	62	535	5,650	18	6	75	1	0
36	♀	4.8	2	1.0520	7.68	1.33	14.42	18.7	39.5	51.3	36.3	59	65	63	60	450	7,900	19	3	78	0	0
37	♂	6.8	2		5.39	3.00	9.80	18.1	27.5	50.8	35.6	36	50	46	40	1205	17,150	12	2	86	1	1
38	♂	7.4	2		7.27	1.00	12.80	16.9	36.7	50.5	33.5	69	64	60	55	930	8,012	14	2	84	0	0
39	♂	7.4	2		7.27	2.49	14.51	19.9	41.0	56.4	35.5	69	62	70	61	775	13,150	14	3	83	0	0
40	♀	6.0	2		7.03	4.00	15.20	21.6	42.0	59.7	36.1	72	68	64	59	770	14,150	28	2	69	1	0
41	♂	8.2	2		6.78	2.00	15.59	22.9	39.0	57.5	39.5	35	63	60	60	660	11,750	15	2	81	1	1
42	♂♀	10.4	2		6.98	3.00	13.90	19.9	39.0	55.8	35.6	26	70	68	59	720	6,300	16	0	80	1	1
43	♀	11.4	2		5.62	2.00	12.60	20.6	32.0	58.8	35.0	48	58	53	47	540	8,550	22	0	73	5	0
44	♂♀	12.3	2		7.32	1.00	15.20	20.7	39.0	53.2	38.9	33	64	60	55	610	8,400	15	2	81	2	0
45	♀	14.0	2		6.86	2.60	13.32	19.4	40.0	58.3	33.2	20	60	63	52	1020	12,000	14	0	85	0	1
46	♂	11.8	2		6.72	2.00	15.20	22.6	40.0	59.5	37.9	20	68	63	58	410	6,400	15	3	80	1	1
47	♂	6.4	3		5.54	2.77	11.59	21.1	36.3	66.0	32.0	57	58	51	46	810	8,110	23	1	75	1	0
48	♀	6.4	3		5.87	2.33	9.37	15.9	27.3	46.5	34.1	39	46	44	39	746	6,933	24	1	75	0	0
49	♀	15.4	4		6.48	3.22	12.90	20.0	35.5	55.0	36.1	61	60	56	49	587	9,987	17	1	81	1	0
50	♀	9.6	4		6.71	3.00	14.02	20.4	39.3	57.6	35.6	48	67	64	58	587	8,100	17	1	81	1	0
51	♀	8.5	4	1.0450	5.84	2.66	12.52	21.5	33.5	57.7	37.4	48	74	58	49	447	7,343	22	2	79	2	0
52	♂	9.0	5	1.0536	6.27	2.16	13.42	20.0	37.5	60.6	33.0	23	57	58	56	592	12,090	26	1	70	2	0
53	♂	15.6	5	1.0538	6.22	2.20	12.82	20.4	37.6	60.4	33.4	23	57	59	56	588	12,090	22	1	76	1	0
54	♂	9.0	3	1.0560	6.99	2.00	15.99	21.8	44.7	66.8	34.6	56	71	70	68	860	12,533	26	9	62	3	0
55	♀	11.8	3	1.0560	7.62	2.00	14.37	18.8	48.0	62.8	30.7	35	65	64	61	653	10,958	21	1	76	1	0
56		9.0	3	1.0500	6.39	2.77	13.89	20.6	48.0	71.8	29.8	55	63	54	51	470	8,133	14	2	84	0	0
57	♀	9.0	2	1.0557	6.28	3.00	13.32	21.1	38.5	60.1	33.3	58	68	54	58	755	9,620	21	1	78	0	0
58	♂	9.0	2	1.0551	6.20	3.00	14.11	21.1	40.0	59.7	35.3	76	72	63	50	680	10,850	29	1	70	0	0
59	♂	6.0	2	1.0527	6.11	1.00	12.96	21.2	38.0	62.2	34.0	52	62	57	52	500	9,100	9	1	89	1	0
60	♀	7.0	3	1.0500	6.68	1.00	15.60	23.4	48.0	72.7	30.6	15	59	60	58	727	7,050	18	2	78	2	0
Average				1.0503	6.16	1.85	13.01	20.0	38.6	59.3	34.3	43	63	59	54	621	11,165	20	4	74	2	0

found an average of 5,973,739 red blood cells, with extremes of 4.63 and 7.76 million. Wells and Sutton ('16), on four two-year-old animals, born and bred in their kennels, found an average of 6.71 million and extremes of 5.6 to 7.6 million. Emmons ('27) gives 6.25 million as the average count in the dog. Pierce and Plant ('28) found counts ranging from 5.4 to 6.73 million in sixteen dogs. Powers, Bowie, and Howard ('30) studied twenty-five male dogs and found the red-cell count to vary between 5.07 and 8.61 million, the average count being 7.00 million. The variations in the count from the aver-

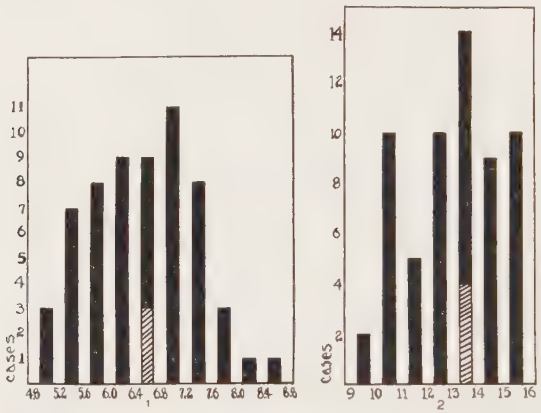

Fig. 1 Average red-cell counts in sixty normal dogs.
Fig. 2 Average Hb values in sixty normal dogs.

age over a short period is usually not more than 4 per cent. For longer periods, it is greater. Thus fifteen weekly counts on dog 9 showed fluctuations from 4.6 to 6.4 million, with an average of 5.54 million for the period; dog 7 over a period of five weeks showed counts from 4.7 to 6.1 million and an average of 5.93 million. Reticulocyte counts show very little variation in different animals. Some of them are usually found in each smear. The average figure of 1.85 per cent is equivalent to 113,960 reticulated cells. Musser and Krumbhaar ('14) found that the percentage varied in eleven dogs between total absence and 1.4 per cent.

Pierce and Plant ('28), using the Dare hemoglobinometer, obtained values corresponding to from 14 to 15.5 grams of Hb per 100 cc. in eleven animals, with two animals showing 12 to 13 grams. Emmons ('27) obtained a value corresponding to 15.48 grams per 100 cc., using a Sahli hemoglobinometer. Musser and Krumbhaar ('14), using the von Fleischl hemoglobinometer, also found 15.48 grams per 100 cc. as the average value for forty-seven dogs, the extremes being 13.80 and 16.38 grams. In none of these cases is any mention made as to whether the instruments had been calibrated. Stucky and Rose ('29), using the Cohen-Smith method, found an average of 15.04 grams per 100 cc., with extremes of 11.32 and 17.53 grams in twelve dogs fed the Cowgill diet. Powers, Bowie, and Howard ('30) obtained a value corresponding to 15.96 grams per 100 cc., using the Sahli method. Our average figure is somewhat lower than these, although 72 per cent of our values fall between 12 and 16 grams and 32 per cent fall between 14 and 16 grams (fig. 2). It may be true that dogs in this semitropical environment have a lower hemoglobin content than do animals in other climates, although Wintrobe and Miller ('29) found the average Hb for one hundred men to be similar to that of observers in other parts of the world. The fluctuations over a period of time in individual animals usually follow those in the reds, but it is to be remarked that with both the diets used the level of Hb can be kept unusually steady. The average figure for corpuscular Hb in this series is somewhat lower than that of 24.0×10^{-12} grams given by Drastich ('28), using only two dogs. The level is surprisingly constant in each animal. Thus in dog 9, for a period of fifteen weeks, the extremes were 21.3 and 25.2×10^{-12} grams, with an average of 23.3×10^{-12} grams. Horneffer ('28) found this value to be so constant in an examination of forty human cases that he suggests that the number of red cells can be calculated from it if the total Hb content is known.

The hematocrit readings show a wide distribution, but 90 per cent of them are between 31 and 49 per cent (fig. 3). Bennett ('26), using Deland hematocrits, obtained values

ranging from 31 to 41 per cent on five normal dogs. Emmons ('27), using the same method, gives 43 per cent as the normal figure. Both of these observers used 'oxalated blood,' with no mention of the amount of oxalate used. Powers, Bowie, and Howard ('30) obtained hematocrit values of 38.2 to 55 per cent in their study of twenty-five dogs. Their average value was 45.2 per cent. The hematocrit level remains relatively constant over periods of weeks. Dog 9 showed fluctuations of from 38.5 to 42.5 per cent over a period of thirteen

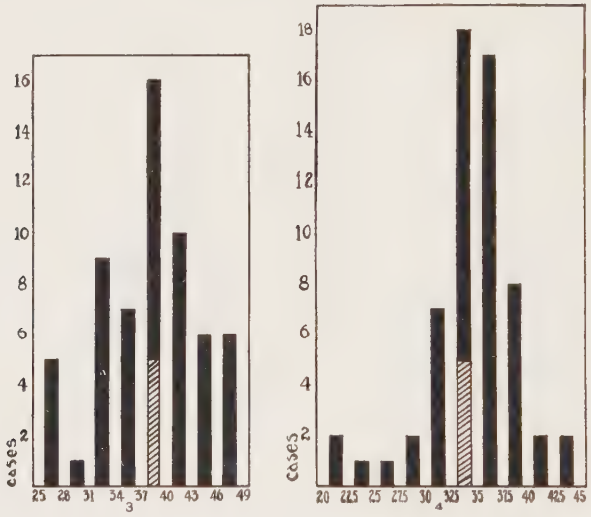

Fig. 3 Average hematocrit values for sixty normal dogs.
Fig. 4 Percentage of Hb in the average red cell in sixty normal dogs.

weeks and an average of 39.0 per cent; dog 7, from 30 to 38.5 per cent during five weeks and an average of 32.4 per cent. The average corpuscular volume for the sixty animals is 59.3 cu.μ. Bodansky and Dressler ('27) obtained an average of 50.7 on seven dogs and reported that animals having a higher count showed a tendency to smaller cells. This does not show in this series. Emmons ('27) gives 69.0 cu.μ as the normal figure for corpuscular volume in dogs. The average corpuscular volume calculated from the data of Powers, Bowie, and Howard ('30) is 64.5 cu.μ.

The per cent saturation of the red cells is even more constant than is corpuscular Hb for the individual animal and for the group. Figure 4 shows that 80 per cent of the values are between 30 and 40 per cent and 58 per cent are between 32.5 and 37 per cent. This constancy is particularly significant, in view of the relatively greater variation in the functions from which the value is calculated, and indicates a nice adjustment for the maintenance of an important 'steady state,' that is to say, the amount of Hb in each cell, or, in other words, the amount of O_2 carried by the blood. Drastich

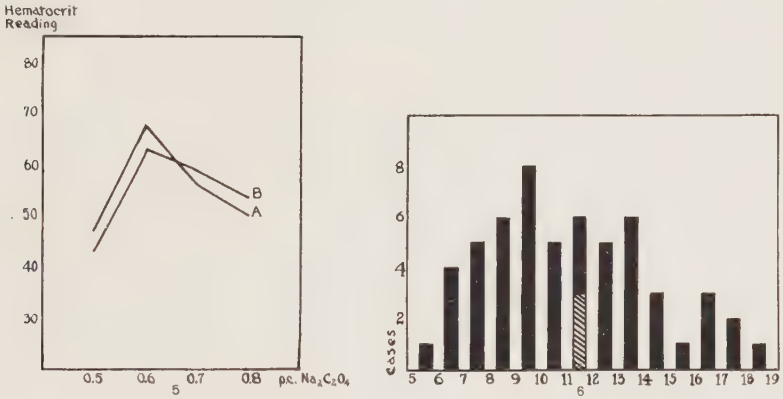

Fig. 5 Average fragility curves for man (A) and dog (B).
Fig. 6 Average white-cell counts in sixty normal dogs.

('28) found not only consistent values of Hb saturation for men and women, but a good agreement for man, dog, and rat. The determinations of corpuscular Hb and per cent saturation should therefore be particularly valuable indices in differentiating various types of anemias, as has been suggested by Haden ('23 b) and by Osgood ('26).

The resistance of the red cells to hypotonic solutions of sodium oxalate varies considerably in different animals, but is relatively constant in the same animal. The results in the table are expressed as hematocrit readings in per cent. The average curve is similar to that given by Silvette ('28) for man (fig. 5). The average number of platelets is found to

be 617,000, with extremes of 370,000 and 1,205,000. Fifty-one per cent of the values are between 450,000 and 600,000. Occasional animals (29 and 37) show exceedingly high values which persist. These values have no relation to the red-cell count. Loesch, Witts, and Zimmermann ('24) found about 500,000 as the normal for dogs, while Krueger and Schultz ('25) found values from 380,000 to 570,000. The leucocytes, as would be expected, vary considerably in the same animal and in different animals. The average count is 11,165, with extremes of 5,650 and 19,200. Musser and Krumbhaar ('14) found an average of 15,923, with extremes of 8,800 and 33,050 in twenty-four dogs. Wells and Sutton ('15) found them to vary between 7,000 and 12,000 in five dogs; Loesch, Witts, and Zimmermann ('24), between 7,000 and 20,000; and Pierce and Plant ('28), from 8,700 to 17,900 in sixteen dogs, in three dogs consistently running above 13,500. The distribution of our average is indicated in figure 6. Values of 8,000 to 14,000 are common and 68 per cent lie between 7,000 and 14,000. The average differential count is similar to that of man and to that given by Musser and Krumbhaar ('14) and by Pierce and Plant ('28) for the dog. The maximum variations for fifteen weeks in dog 9 were as follows: S = 15–20; L = 1–5; N = 68–77; E = 3–8; B = 0–1.

SUMMARY

A study of the blood picture of sixty normal dogs under standard conditions gives the following average figures: *specific gravity* = 1.0503; *red blood cells* = 6.16 million; *reticulocytes* = 1.85 per cent of the red-cell count (113,960); Hb = 13.01 grams per 100 cc.; *corpuscular Hb* = 20.0 grams $\times 10^{-12}$; *hematocrit* = 38.6 per cent; *corpuscular volume* = 59.3 cu.μ; *saturation per cent* = 34.3; *platelets* = 621,000; *white blood cells* = 11,165. The average differential count is 20 per cent small mononuclears, 4 per cent large mononuclears, 74 per cent neutrophiles, and 2 per cent eosinophiles. Basophiles are found only occasionally. Average values are also given for the resistance of the red blood cells to hypotonic sodium-

oxalate solutions. The significance of these average figures is indicated and the variations in the various constituents over short periods of time are mentioned.

It is a pleasure to acknowledge the generous aid of Miss Irene Sellier in the collecting and recording of the data here presented.

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A NOTE ON THE MEGAKARYOCYTES OF THE NORMAL CAT'S SPLEEN

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THREE PLATES (NINE FIGURES)

Despite numerous investigations upon the giant cells of the blood-forming organs, comparatively little work has been done on those cells found in the postnatal spleen. Megakaryocytes have, indeed, been frequently noted in the spleen of various mammals, but little emphasis has been laid upon their occurrence, development, and possible significance. In the bone marrow, on the other hand, this cell type has received much attention. This has undoubtedly been due to the fact that these large cells are uniformly found in greater numbers in the red bone marrow and offer, consequently, a broader field for investigation. Those familiar with the literature on megakaryocytes know that they have been observed and studied in the embryonic mammalian spleen—a site of blood-cell formation. In a recent contribution Hartmann ('30), in noting their presence in the foetal spleen, goes a step further by specifying ther initial appearance and occurrence as coincident with that of erythrocyte development.

The fact that giant cells are present in the postnatal spleen has been shown by various investigators. Wright ('05, '10), for example, observes them in cat, mouse, rat, guinea-pig, and opossum; and, again, Hartmann ('30) mentions them as being present in the dog, cat, rabbit, guinea-pig, bat, mouse, dormouse, and rat, and di Guglielmo ('20), in the cat. Only the most recent editions of histological texts, Sabotta ('29), Bremer ('30), Schafer ('29), recognize the megakaryocyte as a cell type commonly found in the spleen of various mam-

mals. Klaschen ('22), however, is one of the few to direct his entire attention to the spleen as an object of special interest for the study of the megakaryocyte.

While examining sections of spleen of the kitten, rat, and guinea-pig for evidences of erythropoiesis, the attention of the writer was frequently drawn to these curious cells, which stood out in striking contrast to their background of splenic tissue.[1] This paper is an outgrowth of the subsequent interest in these cells, in their frequency of occurrence, and their histogenesis.

MATERIAL

Although sections of spleen of the rat, dog, and guinea-pig were studied, the spleen of the cat proved to be particularly good material. Spleens, obtained from kittens one hour old to fully grow cats, were fixed in Helly's fluid, embedded in paraffin, cut serially from 5 to 7 μ, and stained in hematoxylin and eosin, eosin and methylene blue, Wright's, Nocht's-Hastings, and Mallory's connective-tissue stain. Of these, the sections stained with eosin and methylene blue were found to be the most useful.

Some of the spleens were first injected and washed with warm physiological salt solution, followed by warm Helly's fluid. The tissue was then removed from the animal, cut into smaller portions, and immersed in Helly's fluid for about twelve hours, washed, dehydrated, cleared in xylene, embedded in paraffin, sectioned serially from 5 to 7 μ in thickness, and stained in eosin and methylene blue and in Mallory's connective-tissue stain. This tissue afforded excellent material for investigation—particularly in tracing the early development of the megakaryocyte.

OBSERVATIONS AND DISCUSSION

As mentioned in the foregoing portion, the megakaryocytes of the kitten's spleen stand out with a prominence which

[1] This investigation was carried on in the Laboratory of Histology and Embryology at Cornell University. The writer wishes to express her gratitude to Prof. B. F. Kingsbury for his interest and helpful suggestions.

tends to attract the attention of the observer. This picture was typical for the spleens of all young kittens. A subsequent search was therefore made to determine whether or not these cells could be found in the normal cat's spleen throughout the lifetime of the animal. An examination of tissue obtained from more than thirty individuals showed definitely that, with few exceptions, this cell type can be seen in both the young and the adult animal. In presenting the results of this investigation the following points deserve consideration.

1. The megakaryocytes vary uniformly in their frequency of occurrence. They were far more abundant in the spleens of kittens ranging from several weeks to several months than in the newly born kitten or the adult animal. In the former case these cells averaged six to eight to a section and in the latter instance from three to four cells to a section or even less. They were found lying within the pulp, usually in close proximity to a vein, but never within the lymphatic nodules. Frequently groups of giant cells could be observed packed tightly against the wall of a venous channel (fig. 1), while in other cases some were seen lying within the vein itself (fig. 2).

I have previously alluded to the fact that there were a few instances where giant cells could not be found. In those cases, instances of erythrocyte formation were not present. It may be said that in spleens where erythropoiesis occurs to a greater or less degree, megakaryocytes can be seen. It is fitting to mention here that foci of red-blood-cell formation may commonly be observed in the spleen of the kitten. Hartmann ('30) has already noted the presence of giant cells in the embryonic spleen as soon as erythrocytes begin to develop. There seems to be a curious interrelationship existing between erythropoiesis and giant-cell development. Jordan ('25), in studying the red bone marrow and embryonic blood-forming organs, regards these cells as 'multiple erythroblasts,' in that he has seen the cytoplasm break up to form red blood cells. I have not yet been able to observe this formation of erythrocytes by giant cells. He, moreover, derives these 'hemogenic' giant cells from the 'hemoblast'—

a blood-formative cell which he claims originates in the mesenchyme or endothelium. This leads us to a consideration of the second point.

2. An examination of the washed splenic tissue revealed the fact that, at least in the postnatal spleen, the megakaryocyte develops from a large free cell with a basophilic cytoplasm and a large vesicular nucleus. This cell is apparently the haemocytoblast of Maximow ('27), because of its multiple potentialities and because of its origin from mesenchyme in the embryo and from the reticulum, or, more properly, from the reticulo-endothelium during adult life. With the exception of its origin from endothelium, this cell would also correspond to the hemoblast of Jordan—indeed, I believe it to be the same cell, the discrepancy lying in the diverse interpretation of reticulum and endothelium. A discussion of that widely disputed question is beyond the scope of this paper. Klaschen ('22) and Hartmann ('30) also derive the megakaryocyte from a large free lymphocyte cell. Levy ('21) claims the myelocyte as the stem cell. In many cases megakaryocytes were seen developing from megaloblasts or erythoblasts. These cells are, in turn, derivatives of the haemocytoblast. The megakaryocyte may therefore probably be interpreted as belonging to the group of cells of the erythrocyte series.

In sections of washed tissue it is possible to trace the entire development of the megakaryocyte from the haemocytoblast into: 1) A slightly larger mononuclear form (figs. 4, 6, 7). The nucleus here is slightly enlarged and shows a tendency to become lobed. Figure 7 shows the fact that the cell outline itself tends to become irregular at an early period. 2) In figures 5 and 8 a still later developmental stage is represented. The cell shown here contains a typically lobed nucleus. Closer scrutiny reveals the fact that these lobes are sometimes connected by mere threads of nuclear material. The cell body itself is likewise greatly enlarged. The nucleus, which in the majority of cases develops apparently by hypertrophy and subsequent constriction to form the lobes, may

assume various bizarre forms. Figure 8, a drawing of a cell shown in figure 5, represents a hollow, lobed nucleus with a central cavity described by Heidenhain ('07) as the 'pyrenocoel,' containing the endoplasm, which may communicate with the outer exoplasm by canals which pierce the nuclear wall. Mitoses have been observed by some workers—for example, Heidenhain ('07), Bunting ('09), and Maximow ('10)—although Maximow ('10) has found many more instances of amitosis occurring and leans toward the latter process as the more common. A definite atypical mitotic figure was observed by the writer in only one instance. Levy ('21), in working with the red bone marrow, foetal liver and spleen, describes the formation of mitotic figures and the development of several nuclei followed by a fusion of those separate elements to form the lobed nuclear complex of the giant cell. Evidence from the material studied points strongly to the incorrectness of Levy's interpretation.

The idea that giant cells may develop from a fusion of separate cellular elements has been suggested by various tissue-culture workers. In 1921, the Lewises, in studying hanging-drop preparations of blood cells, noticed the development of multinucleated giant cells from leucocytes. Previous to this, Lewis and Webster ('21) note in cultures of lymph nodes a formation of giant cells as the result of a fusion of large mononuclear wandering cells or of an amitotic division of one cell without an accompanying division of cytoplasm. Barta ('25), in studying cultures of lymph nodes developing in plasma diluted with Ringer's solution and distilled water, notes the twofold development of giant cells from migrating cells. In this connection it may be said that in the spleen, at least, the megakaryocyte does not result from the fusion of separate cells.

The amount of cytoplasm associated with a nucleus varies greatly. One finds various instances where granules, vacuoles, and phagocytic inclusions have been seen and described. Only with considerable difficulty have granules been seen in the younger megakaryocytes. In the larger forms the gran-

ules described by Wright ('05, '10) can be seen bounded by a clearer zone of exoplasmic material. Structures suggesting vacuoles were detected in one or two cases only. Reitano ('21) has found phagocytic giant cells in the spleen of the cat. As yet, I have not been able to verify his observations.

Hartmann ('30) quotes instances in which she has found this cell type connected with the reticulum. An examination of the washed tissue for similar pictures yielded negative results. The larger megakaryocyte lying within the reticular network tends to fill entirely, or even expand, the space within which it is lodged. In this event, the cytoplasm of the giant cell can scarcely be distinguished from the reticular tissue. This situation would account for one of Hartmann's figures and her description of cases, in which she found giant cells having connections with the reticulum network.

Although there are small foci of red-blood-cell formation in the spleen of the young kitten, erythropoiesis is not nearly so intense as in the case of the embryo. Throughout both foetal and postembryonic life haemocytoblasts can be observed undergoing atypical growth which results in megakaryocyte formation. This tendency toward the atypical development of megakaryocytes from haemocytoblasts is particularly noticeable in the spleen of the kitten when erythropoiesis appears to be slowing down. A reference was previously made to groups of giant cells which could be seen lying in close proximity to a vein (fig. 1)—in fact, the majority of these cells were located near veins. This observation may possibly prove a clue to the physiological conditions leading to giant-cell development. At this point the work of Barta ('25) is most enlightening. In studying tissue cultures of lymph nodes, Barta finds that those cells lying exposed or near the air surface tend to remain small, while those lying in the deeper layers and away from the air surface tend to form giant cells either through amitosis, without the usual cytoplasmic division, or through the fusion of neighboring cells. Thus, as Barta states, deficient oxidation fosters giant-cell formation. It is significant, therefore, that the great

majority of megakaryocytes of the spleen are to be found near veins, where there is apt to be a greater lack of oxygen than anywhere else. Moreover, the younger cell forms were also found near the splenic veins—suggesting that the stimulus for this development is in all probability due to varying degrees of oxygen deficiency. A condition of this sort is bound to exist to a greater extent in any tissue such as the red bone marrow, spleen, and lymphatic nodes where the blood stream is slowed down to such an extent as to produce stagnation—a condition which appears conducive to blood-cell formation.

3. Another point which claims more than passing comment is the fact that the cytoplasmic border of some of the splenic giant cells had a somewhat frayed-out appearance. Closer examination showed the cytoplasm breaking up into small cytoplasmic fragments which became disc-like in outline. These bodies were undoubtedly blood platelets. In some cases platelets were noticed in the act of pinching off from the remaining cell body (fig. 9). In other cases whole regions of cytoplasm could be seen breaking up into masses of blood platelets of uniform size (figs. 3 and 9). The development of these platelets has been noted in cases where giant cells have extended long pseudopodia into a vein, or where the cell itself actually projects into the vein (figs. 3, 9), or, finally, where cells themselves come to lie within the vein itself. Some few cases of the extravascular liberation of platelets were seen as well. The development of giant-cell pseudopodia in the bone marrow and spleen has already been described by Schridde ('07), Wright ('10), Klaschen ('22), Kaufman ('29), Hartmann ('30), and others. The intravascular projection of the pseudopodia has likewise been seen by the same investigators. It is interesting to note that naked, disintegratory nuclei could be seen in both veins and pulp.

In 1905, Wright first published a paper in which he definitely states that the blood platelets originate from the cytoplasm of the megakaryocyte. Five years later, after a further study of red bone marrow and spleen, he again published

similar views. From that time the origin of the blood platelets has been an open question. To illustrate: Bunting ('09), Ogata ('12), Klaschen ('22), and others tend to support Wright's theory. The more recent work of Petrie ('25) tends to discredit the interpretation of Wright. In his discussion he mentions the platelets as uniformly round or oval bodies—a condition which could easily be seen in the preparations of kitten's spleen. He furthermore finds that after experimental bleeding, injections of saponin, pyrodin, and peptones, and the production of asphyxia by means of oxygen deficiency or through the introduction of illuminating gas, the number of megakaryocytes of the red bone marrow, spleen, and lymph nodes remains about the same, regardless of the fluctuations in the blood-platelet count. He infers, therefore, that there is no interrelationship between giant cell and blood platelet. In his discussion Petrie refers to similar experiments by other workers interested in the same problem, some of whom lean toward his interpretation. He also cites the work of others who, on the contrary, do find a definite relationship between the rise in blood-platelet count and a simultaneous increase in the number of megakaryocytes. Continued work of an experimental nature may eventually lead to significant results. Up to the present time, however, the balance of evidence from both histological and experimental observations suggests strongly that the blood platelets take their origin in the megakaryocytes. This condition is true for the spleen as well as the red bone marrow.

SUMMARY

The following conclusions may be drawn from the foregoing study:

1. Megakaryocytes are normally found in the spleen of the cat. They are found in greater numbers in the spleens of young kittens than in the newly born and adult cats.

2. These cells develop from a free lymphocyte cell—the haemocytoblast—and are interpreted as belonging to the cells of the erythrocyte series.

3. The giant cells of the spleen are usually found lying in close proximity to the venous channels, where there is a greater degree of oxygen deficiency—a condition which possibly furnishes the stimulus for the development of this cell type.

4. Megakaryocytes may be seen projecting into veins or lying loose within the veins themselves. In this location blood platelets may be seen arising as pinched-off, cytoplasmic, fragments of their pseudopodia.

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PLATE 1

EXPLANATION OF FIGURES

1 Photograph of a transection of a kitten's spleen, showing clusters of megakaryocytes lying beside a vein. × 380.

2 Photograph of a transection of a kitten's spleen, showing three megakaryocytes within the veins. × 380.

3 Photograph of a transection of a cat's spleen, showing a giant cell projecting into a vein and giving rise to blood platelets. × 380.

ABBREVIATIONS

g., giant cell
g.v., giant cell projecting into a vein
p., blood platelet
v., vein

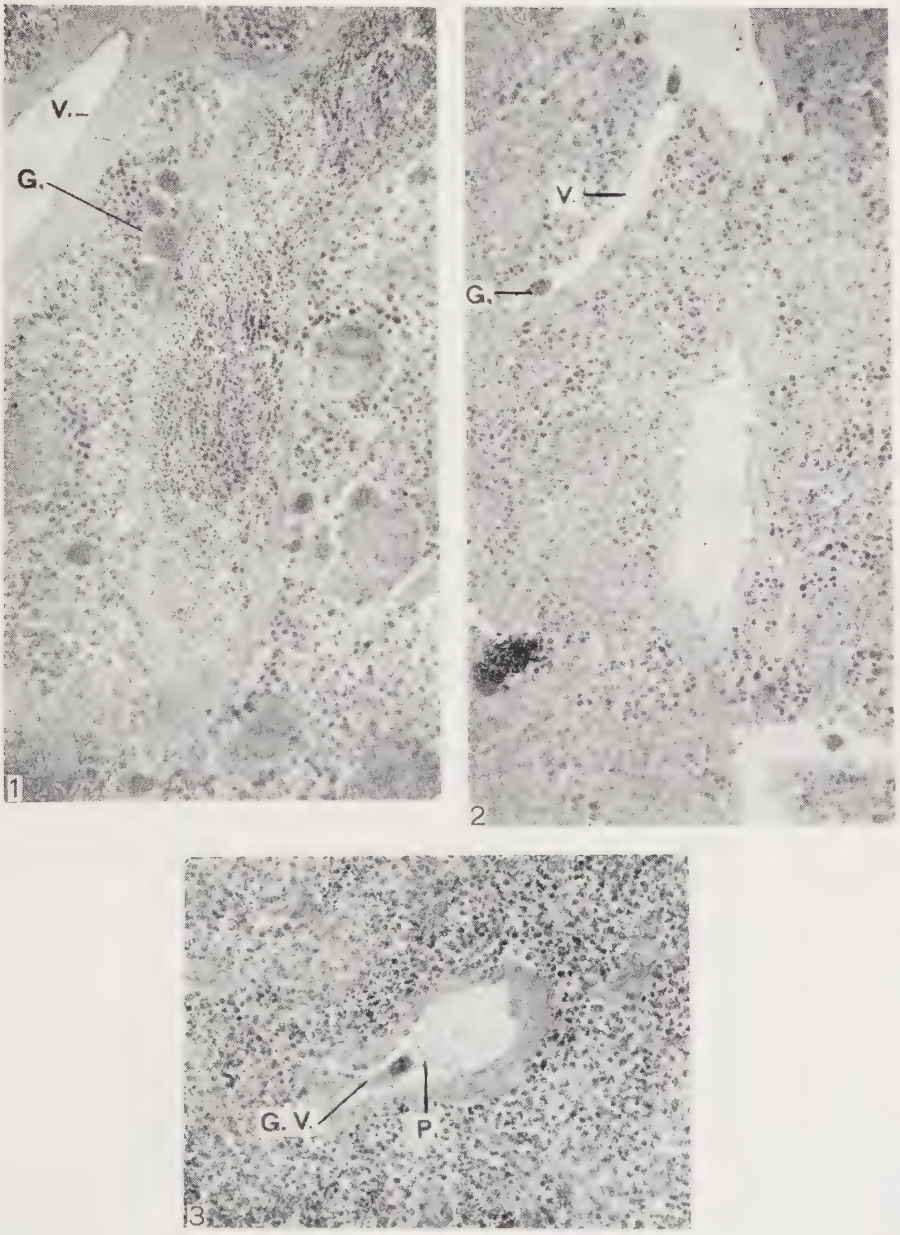

V.
G.
1
V.
G.
2
G. V.
P.
3

PLATE 2

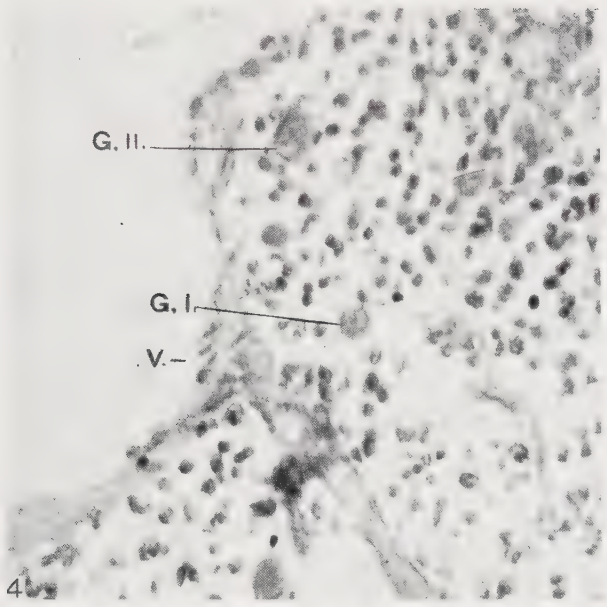
EXPLANATION OF FIGURES

4 Photograph of two young giant cells from the washed spleen of a four-weeks-old kitten. × 450.

5 Photograph of a later stage in the development of a megakaryocyte from the washed spleen of a four-weeks-old kitten. × 450.

ABBREVIATIONS

g., giant cell
g.1., young megakaryocyte
g.2., young megakaryocyte representing a later stage than *g.1.*
v., vein


G. II.
G. I.
V.
4

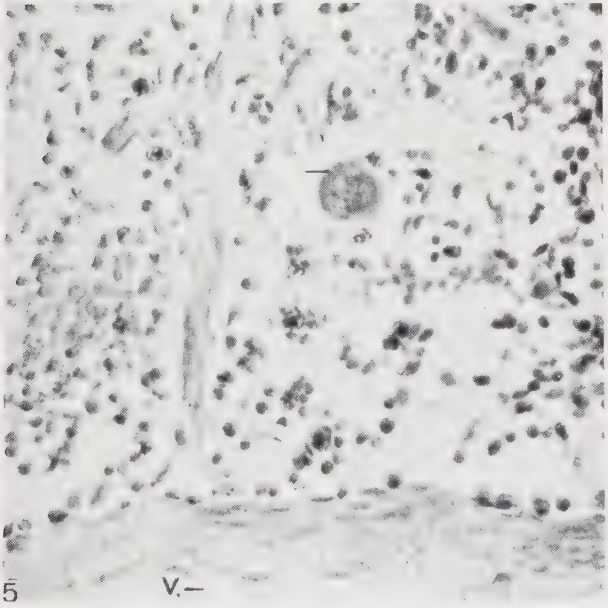

5
V.

PLATE 3

EXPLANATION OF FIGURES

6 Camera-lucida drawing of cell *g.1.*, shown in figure 4. × 1033.

7 Camera-lucida drawing of cell *g.2.*, shown in figure 4. × 1033.

8 Camera-lucida drawing of giant cell shown in figure 5. × 1033.

9 Camera-lucida drawing of giant cell giving off blood platelets, shown in figure 3. × 1033.

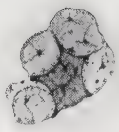
ABBREVIATIONS

g.v., giant cell projecting into vein

p., blood platelets

6

7



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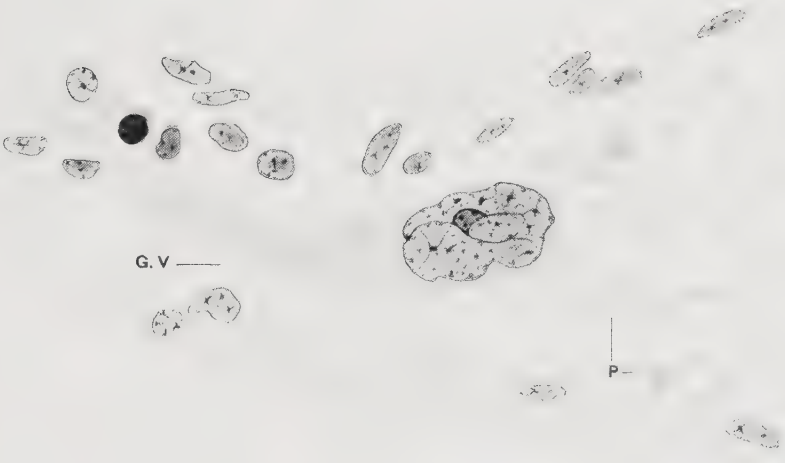

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NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

BLUTUNG UND FLUOR, by Prof. Dr. Hans Runge, Foreword by Prof. Dr. Robert Schröder. (Medizinische Praxis, Sammlung für ärztliche Fortbildung, Band IX.) 1930. Size, 6 × 8¾ inches. 20 illustrations. Pages x + 108. Index. Price, RM 8; bound, RM 9.50. Theodor Steinkopf, Dresden-Blasewitz.

From the Woman's Clinic of the University of Kiel has come this book designed to aid the gynecologist and the general practitioner. It discusses kinds of hemorrhages and discharges and their significance, and gives specialized information for diagnosis and treatment, by modern methods, of the diseases of which these are symptoms.

ARBEITEN AUS DEM NEUROLOGISCHEN INSTITUTE (österr. interakademisches Zentralinstitut für Hirnforschung) AN DER WIENER UNIVERSITÄT. XXXII. Band. 1930. Size, 7 × 10 inches. 342 pages. 108 illustrations. Bibliographies. Price, M 70. Franz Deuticke, Wien und Leipzig.

The twelve original articles making up this volume are as follows: Über die Struktur der Nervenfaser, by L. Jaburek; Der Rollersche Kern, by Tameo Nakamura; Kohlenoxydvergiftung und Zentralnervensystem, by E. Pollak and Ph. Rezek; Zur Pathologie der Ganglienzellen bei multipler Sklerose, by Grete Zellman; Die Todesursache bei multipler Sklerose, by Grete Zellman; Der Einfluss labyrinthärer und kortikaler Reizung auf die Augenstellung nach Durchschneidung des hinteren Längsbündels, by E. A. Spiegel and Lad. Tokay; Der Parkinsonismus symptomaticus, by Eugen Brzezicki; Studien über die Chorea chronica und die Beziehung des Striatum zu dieser, by Ladislaus Tokay; Untersuchungen über die Veränderungen des zentralen Nervensystems bei Septiko-Pyämie, by Gisaku Kobayashi; Vergleichend-anatomische Untersuchungen über den sogenannten akzessorischen Abduzenskern, by Tameo Nakamura; Zur Frage der traumatischen Hämorrhagie, by Gisaku Kobayashi; Über den Nucleus triangularis, by W. J. Godlowski.

MEMORIA ANUAL DE 1924, MUSEO NACIONAL DE HISTORIA NATURAL, BUENOS AIRES, by M. Doello-Jurado. 118 pages of text. 44 plates. Size, 7 × 10½ inches. Index.

This volume was published in commemoration of the founding of the Museum, and its hundred years of existence (1823–1923). It describes the organization, the accomplishments, the current work, and the future plans.

THE ROCKEFELLER FOUNDATION ANNUAL REPORT FOR 1929. Size, 5¾ × 8¾ inches. 402 pages. Illustrated. Indexed. The Rockefeller Foundation, New York.

Contains the President's Review; The Reports of the Secretary and the Treasurer; Reports of the work of the International Health Division, and the work done in the Medical Sciences, the Social Sciences, and the Humanities.

PROGRAM AND ABSTRACTS FOR THE TWENTY-EIGHTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOÖLOGISTS

CLEVELAND, OHIO, DECEMBER 30, 31, 1930, AND JANUARY 1, 1931

These titles and abstracts make up the 1930 program of the American Society of Zoölogists. They were received on or before November 15th either by the Secretary of the Society, or by the Secretary of the Joint Genetics Sections of the Society and the Botanical Society of America, and have been arranged according to the accepted program rules.

The abstracts are in the form contributed by their authors. They have been edited only to secure a uniform system of titles and style and to reduce length when obviously overlong. Their arrangement and the planning of the program can be done only after the titles are all at hand and must be greatly rushed in order to obtain publication before the meetings. The Wistar Institute is scheduled to mail out this issue of The Anatomical Record three weeks, to the day, after receiving the manuscript. All proofreading will be done there. The Institute deserves great credit for making possible the early publication and distribution of this material.

These abstracts and the accompanying data are presented as a part of the official proceedings of the 1930 meetings of the Society. The minutes of these meetings and a list of the officers of the Society for 1931 will appear in the January number of this journal.

THE ANATOMICAL RECORD, VOL. 47, NO. 3
DECEMBER, 1930

PROGRAM

An alphabetical author index of all persons contributing to the program will be found on pages 366–370. Data concerning rooms, not given here, may be found in the program of the American Association for the Advancement of Science.

All sessions of the American Society of Zoölogists will be held in buildings of the Western Reserve University. The room assignments for various sections are as follows:

Tuesday, December 30th

10.00 A.M. *a.* Comparative and General Physiology. Papers 1 to 18, inclusive. Amphitheater, Babies' Hospital.

b. Embryology. Papers 86 to 99, inclusive. Room 234, School of Medicine.

c. Joint session with the American Society of Parasitologists. Papers 179 to 188, inclusive, and papers of general interest to zoölogists by members of the Society of Parasitologists. Amphitheater, Pathological Institute.

d. Joint Genetics Sections. Lecture Room, Biological Laboratory.

2.00 P.M. *a.* Demonstrations and exhibits. Presentation of all papers listed "By demonstration." Room 237, Histological Laboratory, School of Medicine.

8.00 P.M. Biologists' Smoker. Cleveland Museum of Natural History, 2717 Euclid Avenue. All biologists are cordially invited.

Wednesday, December 31st

10.00 A.M. *a.* Comparative and General Physiology. Papers 19 to 31, inclusive. Amphitheater, Babies' Hospital.

b. Cytology. Papers 113 to 122, inclusive. Room 234, School of Medicine.

c. Joint session with the Ecological Society of America. Papers 169 to 174, inclusive, and papers of general interest to zoölogists by members of the Ecological Society of America. Room 33, Main Building, Adelbert College.

d. Joint Genetics Sections. Lecture Room, Biological Laboratory.

2.00 P.M. *a.* Business session of Section F. Room 234, School of Medicine.

2.10 P.M. Business session of the American Society of Zoölogists. Room 234, School of Medicine.

3.00 P.M. Protozoölogy and Miscellaneous. Papers 145 to 148, inclusive, and 158 to 161, inclusive. Room 234, School of Medicine.

2.00 P.M. *b*. Joint Genetics Sections. Demonstrations and exhibits. 30 Biological Laboratory.

6.30 P.M. Zoölogists' Dinner. Hollenden Hotel. The dinner address will be given by W. A. Riley, Vice-President of Section F, on "Some present-day problems in zoölogical teaching." All biologists are cordially invited.

THURSDAY, JANUARY 1ST

10.00 A.M. *a*. Comparative and General Physiology. Papers 32 to 46, inclusive. Room 234, School of Medicine.

b. Joint Genetics Sections. Lecture Room, Biological Laboratory.

LIST OF TITLES

COMPARATIVE AND GENERAL PHYSIOLOGY

Read

1. Locomotion and response in Difflugia. S. O. Mast, Johns Hopkins University. (8 min.)
2. Response in Amoeba to localized photic stimulation. S. O. Mast, Johns Hopkins University. (7 min.)
3. The rate of light adaptation in Eristalis tenax. William L. Dolley, Jr., University of Buffalo. (Lantern; 15 min.)
4. The activating influence of light upon certain aquatic arthropods. William C. Stehr, Ohio University. (Introduced by D. E. Minnich.) (Lantern; 5 min.)
5. The nature of the visual cells of lampreys. G. L. Walls, University of Michigan. (Introduced by A. F. Shull.) (Lantern; 15 min.)
6. The effect of darkness and temperature on the excised eye of the frog. L. B. Arey and W. K. Jennings, Northwestern University Medical School. (Lantern; 10 min.)
7. The effects of x-rays on regeneration in Tubifex. R. G. Stone, University of Missouri. (Introduced by Winterton C. Curtis.) (Charts; 7 min.) (Also by demonstration.)
8. The effects of x-rays on regeneration in the nemertean, Lineus socialis. K. B. Coldwater, University of Missouri. (Introduced by Winterton C. Curtis.) (Charts; 7 min.) (Also by demonstration.)
9. Ultraviolet point radiation and the heart beat of Fundulus, frog, and turtle. M. A. Hinrichs, I. T. Genther, P. O. C. Johnson, and I. Milliken, University of Chicago. (Lantern; 8 min.)
10. Ultraviolet point radiation and the heart beat of Limulus. Marie A. Hinrichs and Ida T. Genther, University of Chicago. (Lantern; 7 min.)
11. Pigment migration in the vermilion-spotted newt induced by ultraviolet radiation. H. H. Collins and E. A. Wolf, University of Pittsburgh. (Lantern; 8 min.)
12. Regeneration and metabolism experiments with electromagnetic fluxes. R. A. Muttkowski and Leo E. Buss, University of Detroit. (Lantern; 7 min.)
13. On the reactions of oyster larvae to currents and to salinity gradients. T. C. Nelson and E. B. Perkins, Rutgers University. (Lantern; 15 min.)
14. The sensitivity of the legs of the monarch butterfly, Anosia plexippus Linn., to saccharose and lactose. Almeda Anderson, University of Minnesota. (Introduced by D. E. Minnich.) (Lantern; 5 min.)
15. The contact chemoreceptors of the honeybee, Apis mellifera Linn. Dwight Elmer Minnich, University of Minnesota. (Lantern; 5 min.)
16. Group learning in goldfish. Carl Welty, University of Chicago and Parsons College. (Introduced by W. C. Allee.) (Lantern; 15 min.)
17. Weight and metabolism changes during the metamorphosis of the Japanese beetle (Popillia japonica Newman). Daniel Ludwig, New York University. (Introduced by the Secretary of the Society.) (Lantern; 10 min.)

18. The developing grasshopper egg. Eleanor H. Slifer, State University of Iowa. (Introduced by J. H. Bodine.) (Lantern; 15 min.)
19. Heart-beat reversal in a crane-fly. John H. Gerould, Dartmouth College. (Lantern; 10 min.)
20. The functions of the tracheal gills in larvae of the caddis fly, Macronema zebratum Hagen. Ann H. Morgan and Helen D. O'Neil, Mount Holyoke College. (Presented by Helen D. O'Neil; introduced by A. E. Adams.) (Lantern; 15 min.)
21. Respiration of the puffer fish. F. G. Hall, Duke University. (Lantern; 12 min.)
22. A physiological study of gastric motility in the crab, Cancer productus. T. L. Patterson, Detroit College of Medicine and Surgery and The Hopkins Marine Station, Stanford University. (Lantern; 15 min.)
23. The mechanism of deposition of calcium to form extraskeletal bone in the adult animal—a preliminary study. Clay Ray Murray and Margaret R. Murray, Columbia University. (Lantern; 15 min.)
24. Histological changes in the maxillary-sinus mucosa following irrigations with calcium-precipitating substances. W. F. Wenner, Washington University. (Lantern; 15 min.)
25. The symbiosis between the wood-feeding roach Cryptocergus punctulatus Scudder and its intestinal flagellates. L. R. Cleveland, Harvard University Medical School. (Lantern; 15 min.) (Also by demonstration.)
26. The relations of numbers of animals to survival time in toxic concentrations of electrolytes. J. R. Fowler, University of Chicago and University of Louisiana. (Introduced by A. E. Emerson.) (Lantern; 15 min.)
27. A study of magnesium anesthesia. L. V. Heilbrunn, University of Pennsylvania. (Lantern; 8 min.)
28. The action of sodium, potassium, and calcium ions on the protoplasmic viscosity of Amoeba dubia. L. V. Heilbrunn and Kathryn Daugherty, University of Pennsylvania. (Lantern; 7 min.)
29. Molecular organization of the protoplasm of amebas. A. A. Schaeffer, University of Kansas. (Lantern; 15 min.)
30. Studies on immunity to rattlesnake venom (crotalin). J. Markowitz, H. E. Essex, and F. C. Mann, Mayo Foundation. (Lantern; 15 min.)
31. The treatment of snake bite. Albert M. Reese, West Virginia University. (10 min.)
32. Artificial insemination with motile sperm from ovariotomized fowl. L. V. Domm, University of Chicago. (Lantern; 8 min.)
33. Implantation of juvenile testicular tissue into the hypertrophied right gonad of ovariotomized fowl. L. V. Domm, University of Chicago. (Lantern; 7 min.)
34. Determination and physiology of Bidder's organ. Emil Witschi, State University of Iowa. (Lantern; 15 min.)
35. Functional interrelations of anterior-hypophysial and gonad hormones. Carl R. Moore and Dorothy Price, University of Chicago. (Charts; lantern; 15 min.)

36. The gonad-stimulating and the luteinizing hormones of the anterior lobe of the hypophysis. H. L. Fevold, Frederick L. Hisaw, and S. L. Leonard, University of Wisconsin. (Introduced by M. F. Guyer.) (Lantern; 15 min.)
37. The function of follicular and corpus-luteum hormones in the uterus of castrate monkeys (Macacus rhesus). F. L. Hisaw, H. L. Fevold, and R. K. Meyer, University of Wisconsin. (Lantern; 15 min.)
38. The potency of the anterior-pituitary sex hormone of normal and unilateral-castrated albino rats. Frederick E. Emery, University of Buffalo. (Introduced by the Secretary of the Society.) (Lantern; 10 min.)
39. Ovariectomy and corpus-luteum-extract experiments in pregnant rats. George E. Johnson and Joanna S. Challans, Kansas State Agricultural College. (10 min.)
40. The reaction of chicks and immature fowls to the sex hormones. Mary Juhn and R. G. Gustavson, University of Chicago and University of Denver. (Introduced by Frank R. Lillie.) (Lantern; 15 min.)
41. Integumental grafting as a means of analyzing the factors determining the secondary sexual characters of the domestic fowl. A. W. Kozelka, University of Chicago. (Introduced by Sewall Wright.) (Lantern; 15 min.)
42. Temperature and male production in the cladoceran Moina macrocopa. L. A. Brown, George Washington University, and Arthur M. Banta, Brown University and Carnegie Institution of Washington. (Lantern; 15 min.)
43. Induced hyperthyroidism and sterility in rats. Bert Cunningham and C. W. Hooker, Duke University. (5 min.)
44. The hormone of the suprarenal cortex. W. W. Swingle and J. J. Pfiffner, Princeton University and Cold Spring Harbor, Long Island. (Lantern; 15 min.)
45. The influence of ferrous iodide and linoleic acid on rats depleted of vitamin A. F. E. Chidester, A. G. Eaton, and N. K. Speicher, West Virginia University. (Lantern; 10 min.)
46. Body size as a factor in the metamorphosis of frogs. Edward F. Adolph, University of Rochester. (Lantern; 12 min.)

By demonstration

47. The surface precipitation reaction in the egg of Chaetopterus. D. P. Costello, University of Pennsylvania. (Introduced by L. V. Heilbrunn.)
48. The action of ultraviolet rays on Paramecium protoplasm. R. A. Troisi, University of Pennsylvania. (Introduced by L. V. Heilbrunn.)
49. Muscular differentiation in oysters exposed for diverse periods of time. Hoyt S. Hopkins, New York University.
50. Rate of growth and body size in relation to the metamorphosis of frogs. Edward F. Adolph, University of Rochester.
51. A biological method of determining circulation time. Theodore Koppányi, Georgetown University School of Medicine.
52. Withdrawn.
53. A demonstration of a working model of the compound eye. Edgar Altenburg, Rice Institute.

By title

54. Effects of white, green, and red lights of equal luminous intensity on the testis activity of the European starling, Sturnus vulgaris. Thomas Hume Bissonnette, Trinity College.
55. Effects of white light of different intensities upon the testis activities of the European starling, Sturnus vulgaris. Thomas Hume Bissonnette, Trinity College.
56. Hydroid pigments. Nellie M. Payne, Marine Biological Association, Plymouth, England. (Introduced by the Secretary of the Society.)
57. Acclimatization to low temperatures in certain marine hydroids. Nellie M. Payne, Marine Biological Association, Plymouth, England. (Introduced by the Secretary of the Society.)
58. Oxygen concentration and oxygen consumption in solutions of different osmotic pressure. J. William Buchanan, Northwestern University.
59. Stimulation in Balanus and Sagartia by normal primary aliphatic aldehydes and acids. William H. Cole, Rutgers University.
60. Physiological significance of H-HS for the development of sea-urchin eggs. T. P. Sun, State University of Iowa. (Introduced by J. H. Bodine.)
61. Inhibition of regeneration in two-headed planarians. E. D. Goldsmith, Harvard University and New York University. (Introduced by H. W. Rand.)
62. Production of supernumerary heads by radial incisions in Planaria maculata and Phagocata gracilis. E. D. Goldsmith, Harvard University. (Introduced by H. W. Rand.)
63. A study of the innervation of the heart of fish embryos. Floyd J. Brinley, North Dakota State College.
64. Migration of retinal pigment in Crustacea in relation to oxygen deficiency. Rudolf Bennitt and Amanda Dickson Merrick, University of Missouri. (Introduced by the Secretary of the Society.)
65. Some observations with the microscope centrifuge. E. Newton Harvey, Princeton University.
66. Irradiation of the ovaries of guinea-pigs and its effect on the oestrous cycle. Ida T. Genther, Washington University. (Introduced by the Secretary of the Society.)
67. The intracellular digestion of thymus nucleoprotein in triclad flatworms. Edward G. Kelley, New York University. (Introduced by H. W. Stunkard.)
68. Some physiological effects of ultraviolet radiation on honeybees. L. M. Bertholf, Western Maryland College, and Bee Culture Laboratory, U. S. Bureau of Entomology. (Introduced by S. O. Mast.)
69. Reactions to light and temperature in the cercariae of the trematode Bucephulus elegans. Gerrit Bevelander, Johns Hopkins University. (Introduced by S. O. Mast.)
70. A quantitative study of reactions to electricity in Amoeba. William F. Hahnert, Johns Hopkins University. (Introduced by S. O. Mast.)
71. A factor modifying growth of head furnishings in Leghorn fowl. L. V. Domm, University of Chicago.
72. Spur dichotomy in the ovariotomized Brown Leghorn fowl. L. V. Domm, University of Chicago.

73. A demonstration of equivalent potentialities of right and left testis-like gonads in ovariotomized fowl. L. V. Domm, University of Chicago.
74. Respiratory metabolism during pupal development of Galleria mellonella. Ivon R. Taylor and H. B. Steinbach, Brown University. (Introduced by H. E. Walter.)
75. Effect of injection of anterior-pituitary extract on the thyroids of mice with hereditary dwarfism. George D. Snell, Dartmouth College and Brown University. (Introduced by A. M. Banta.)
76. An experimental study of the factors concerned in mammary growth and milk secretion. W. O. Nelson and J. J. Pfiffner, Washington Square College, New York University and the Biological Laboratory, Cold Spring Harbor. (Introduced by H. O. Haterius.)
77. Anterior-pituitary therapy in spontaneous ovarian dysfunction. W. O. Nelson, Washington Square College, New York University. (Introduced by H. O. Haterius.)
78. The effect of ovarian implants on castration-cell types in the male rat pituitary. H. O. Haterius and W. O. Nelson, Washington Square College, New York University.
79. Vaginal cornification and ovarian blood supply. H. O. Haterius, Washington Square College, New York University.
80. The influence of ferrous iodide and oleic acid on rats depleted of vitamin A. F. E. Chidester, A. G. Eaton, and N. K. Speicher, West Virginia University.
81. The experimental production of pregnancy cells in the pituitary gland of mice. I. The occurrence of pregnancy cells in female mice following continuous anterior-lobe administration. H. O. Haterius and H. A. Charipper, Washington Square College, New York University.
82. The experimental production of pregnancy cells in the pituitary gland of mice. I. The occurrence of pregnancy cells in male mice following continuous anterior-lobe administration. H. O. Haterius and H. A. Charipper, Washington Square College, New York University.
83. The sensitivity of the legs of certain Calliphoridae to saccharose. Selma Crow, University of Minnesota. (Introduced by D. E. Minnich.)
84. The sensitivity of the oral lobes of the proboscis of the blowfly, Calliphora vomitoria Linn., to various sugars. Dwight Elmer Minnich, University of Minnesota.
85. Hydrogen-ion concentration of the amniotic and allantoic fluids of the pig. Charles E. Abromavich, Jr., Johns Hopkins University. (Introduced by the Secretary of the Society.)

EMBRYOLOGY

Read

86. Viability of Physa egg fragments. E. D. Crabb, University of Colorado. (Lantern; 10 min.)
87. A possible relationship between spermatozoon age and foetal abnormalities in the guinea-pig. William C. Young, Brown University. (Lantern; 15 min.)
88. The postembryonic development of mendelian characters in the goldfish Carassius auratus. H. B. Goodrich and I. B. Hansen, Wesleyan University. (Lantern; 15 min.)

89. Origin of the aortic arches in Amphibia. Waldo Shumway, Selma M. Olson, and Otto E. Kugler, University of Illinois. (Lantern; 10 min.)
90. Differential counts of the leucocytes of fetal albino rats. J. E. Kindred, University of Virginia. (Lantern; 15 min.)
91. The sugar content of the blood of the fetal albino rat. E. L. Corey, University of Virginia. (Lantern; 10 min.)
92. Micromoving pictures showing the embryonic circulation. Bradley M. Patten, Western Reserve University, and (by invitation) Theodore C. Kramer, Baldwin Bird Research Laboratory. (Motion-picture projection; 15 min.)
93. Bisexuality and sex differentiation in embryos of the musk turtle, Sternotherus odoratus (Latreille). Paul L. Risley, University of Michigan. (Introduced by Peter Okkelberg.) (Lantern; 10 min.)
94. Studies of living nerves. I. The movements of sheath cells and nerve sprouts, correlated with the process of myelin formation in amphibian larvae. Carl Caskey Speidel, University of Virginia Medical School. (Lantern; 15 min.)
95. Further experimental studies of the opercular structures in Hydroides dianthus. Charles Zeleny, University of Illinois. (Lantern; 10 min.)
96. Tail induction through the hind parts of the neural plate in Triton. H. Bytinski-Salz, Yale University. (Introduced by R. G. Harrison.) (Lantern; 15 min.)
97. The reproductive system in half-grown western axolotls (Amblystoma tigrinum?) following hypophysectomy. R. K. Burns, Jr., and Adrian Buyse, University of Rochester School of Medicine and Dentistry. (Lantern; 15 min.)
98. The occurrence of abdominal organs in the thoracic cavity in the domestic cat. H. H. Collins and Jean MacCreight, University of Pittsburgh. (Lantern; 7 min.)
99. A twin-headed human fetus. R. A. Muttkowski and Leo E. Buss, University of Detroit. (Lantern; 8 min.)

By demonstration

100. Internal relations found in twins and double monsters produced by ultraviolet radiation. Marie A. Hinrichs, University of Chicago.
101. A bovine freemartin. R. K. Burns, Jr., and Adrian Buyse, University of Rochester, School of Medicine and Dentistry.

By title

102. The nervous structure of the annelid ganglion. W. M. Smallwood, Syracuse University.
103. The development of the carp. Study no. 1. The larval life of the carp, with special reference to the development of the intestinal canal. W. M. Smallwood and Mary Lovett Smallwood, Syracuse University.
104. The development of the carp. Study no. 2. The development of the ear and lateral-line organs. W. M. Smallwood and Phyllis Virginia Plyler, Syracuse University.

105. The aortic arches and their derivatives in the embryo porcupine (Erethizon dorsatus). Parke H. Struthers, Syracuse University. (Introduced by W. M. Smallwood.)
106. Effects on the nervous system following the embryonic transplantation of the spinal cord in the anuran, Discoglossus pictus, Otth. Raoul M. May, College de France and Institut Pasteur.
107. Transplantation of cerebral tissue of the newborn rat into the eye of the adult albino rat. Raoul M. May, College de France and Institut Pasteur.
108. The origin of the rete testis in the domestic fowl. J. C. Gray, Western Reserve University.
109. On the process of incorporation and vascularization of an implant by the chorio-allantoic membrane. Lillian O. Henderson, University of Chicago. (Introduced by B. H. Willier.)
110. Gastrulation in thermal-gradient treated eggs of Triturus torosus. Francis G. Gilchrist, Pomona College.
111. Diagnostic stages of metamorphosis in Amblystoma opacum and Amblystoma jeffersonianum. Madeleine P. Grant, Smith College and Radcliffe College. (Introduced by Alden B. Dawson.)
112. Skin grafting in frog tadpoles: Local specificity of skin and behavior of epidermis. Herbert W. Rand and Madeleine E. Pierce, Radcliffe College.

CYTOLOGY

Read

113. A new method used in studies on the living cells of insects. W. J. Baumgartner, University of Kansas. (Lantern; 12 min.)
114. Seasonal cytolysis and regeneration in the Anura. Roy A. Waggener, Carleton College. (Introduced by B. F. Kingsbury.) (12 min.)
115. Some cytoplasmic structures in the male reproductive cells of a pseudoscorpion (Chelanops corticis). Henry G. Nester, Indiana University. (Introduced by F. Payne.) (Lantern; 10 min.)
116. A comparative study of rodent chromosomes. J. C. Cross, University of Texas. (Introduced by T. S. Painter.) (Lantern; 7 min.)
117. The influence of x-rays upon the processes of secretion in the pancreas, observed in the living cell. G. C. Hirsch, Utrecht (Holland). (Introduced by P. W. Whiting.) (Lantern; 15 min.)
118. The structure and reproduction of the parasome or parabasal body in trichomonad flagellates. Harold Kirby, Jr., University of California. (Lantern; 8 min.)
119. The zone of Golgi in the cartilage cells of Necturus. Alden B. Dawson, Harvard University. (Lantern; 10 min.)
120. Carbohydrate metabolism in relation to the mitochondria of the hepatic cell. J. McA. Kater, Washington State College. (Lantern; 15 min.)
121. A consideration of the central body situation in the second cleavage of Ascaris megalocephala. Lloyd C. Fogg, Washington Square College, New York University. (Introduced by Henry J. Fry.) (Lantern; 15 min.)
122. Nuclear behavior during the formation and secretion of the silk in Bombyx mori. Hope Hibbard, Oberlin College. (Lantern; 10 min.)

By demonstration

123. The occurrence of both anatomical (hepatic) and functional (portal) lobules in the liver of the seal. L. B. Arey, Northwestern University Medical School.
124. Chromosomes of three species of Mantidae, Orthoptera. Robert L. King, State University of Iowa.
125. A cytological study on the spinal-ganglion cells of the rat. H. W. Beams, State University of Iowa. (Introduced by J. H. Bodine.)
126. Demonstration of human X-Y sex chromosomes. T. S. Painter, University of Texas.
127. A demonstration of the nuclear changes during the growth and secretion of the silk gland of Bombyx mori. Hope Hibbard, Oberlin College.
128. A demonstration of the intimate penetration of tracheal tubules into the deep cytoplasm of gland cells. Hope Hibbard, Oberlin College.

By title

129. Studies on the gastric epithelium of grasshoppers. B. H. Woodruff, Western Reserve University. (Introduced by J. P. Visscher.)
130. Effects of experimental inanition and recovery on the blood cells of the salamander, Triturus viridescens. H. E. Jordan, University of Virginia.
131. The pigment content of the liver cells of urodeles. H. E. Jordan, University of Virginia.
132. The vacuome of the hypotrichous ciliate, Stylonychia. R. P. Hall, New York University.
133. On the vacuome of the phytomonad flagellate, Chlamydomonas. R. P. Hall and R. F. Nigrelli, New York University.
134. On the vacuome and the neutral-red reaction in Paramecium caudatum. F. W. Dunihue, New York University. (Introduced by R. P. Hall.)
135. On the relation of the vacuome to metabolism in Euglena, Chlamydomonas and Chlorella. R. P. Hall and F. W. Dunihue, New York University.
136. The so-called 'segregation apparatus' of the erythrocyte of frog and Necturus blood. H. W. Beams, State University of Iowa. (Introduced by J. H. Bodine.)
137. The release of follicular colloid from the thyroid of Amblystoma larvae following heteroplastic anterior-pituitary implants. Madeleine P. Grant, Smith College and Radcliffe College. (Introduced by Alden B. Dawson.)
138. The release of follicular colloid from the thyroids of Amblystoma larvae during diagnostic stages of metamorphosis. Madeleine P. Grant, Smith College and Radcliffe College. (Introduced by Alden B. Dawson.)
139. The services of the beaker cells in the skin of Amphibia. J. V. Shankweiler, Cornell University. (Introduced by the Secretary of Section F.)
140. Some results of the application of Feulgen's nuclear reaction to the early stages in Odonate oogenesis. Arthur D. Whedon, North Dakota Agricultural College.
141. Central bodies in reciprocal hybrids of Echinarachnius and Arbacia. Henry J. Fry and Jacob Katz, Washington Square College, New York University.

142. Central bodies during successive cleavages in Echinarachnius eggs. Henry J. Fry and Malvina Schweizer, Washington Square College, New York University.
143. Asters in fertilized Chaetopterus eggs. Sophye Mohel and Henry J. Fry, Washington Square College, New York University.
144. Comments on cell nomenclature according to Gatenby. Hope Hibbard, Oberlin College.

PROTOZOOLOGY

Read

145. Paramoecium infusion histories. II. *a*) Methods of concentration and, *b*) some causes of aggregation. Edgar P. Jones, University of Pittsburgh. (Introduced by Robert T. Hance.) (10 min.)
146. Is Paramoecium geotropic? Edgar P. Jones, University of Pittsburgh. (Introduced by Robert T. Hance.) (5 min.)
147. Studies on the contractile vacuole of Blepharisma undulans. Imogene Moore, Yale University. (Introduced by L. L. Woodruff.) (15 min.)
148. The origin of the several genera of Opalinidae. Maynard M. Metcalf, Johns Hopkins University. (Lantern; 12 min.)

By demonstration

149. The parabasal apparatus and cytoplasmic inclusions of Trichonympha from Termopsis. Harold Kirby, Jr., University of California.

By title

150. Studies on the plates of the fresh-water Ceratium, the so-called Ceratium hirundinella. C. T. Hurst and D. R. Strong, Western State College of Colorado. (Introduced by the Secretary of the Society.)
151. Membranelles of Stenosemella nivalis. Arthur S. Campbell, Saint Mary's College, California.
152. Further studies of the sequence of Protozoa in five plant infusions. W. Byers Unger, Dartmouth College.
153. A mutation of Chilodon uncinatus produced by ultraviolet radiation. Mary Stuart MacDougall, Agnes Scott College.
154. On the morphology and binary fission of Heteronema acus. J. B. Loefer, New York University. (Introduced by R. P. Hall.)
155. Cultural reactions of Chlamydomonas sp. R. F. Nigrelli, New York University. (Introduced by R. P. Hall.)
156. On the vacuome, food vacuoles, and the neutral-red reaction in Vorticella, and the question of a 'complete digestive tract' in ciliates. R. P. Hall and F. W. Dunihue, New York University.
157. Micronuclear variation in Paramecium bursaria. Lorande Loss Woodruff, Yale University.

MISCELLANEOUS

Read

158. The differential rate of discovery of species of genera, small and large. W. H. Longley, Goucher College. (Lantern; 15 min.)
159. The trunk muscles of snakes and their bearing on taxonomy, and phylogeny. Walter Mosauer, University of Michigan. (Introduced by the Secretary of the Society.) (Lantern; 15 min.)
160. Growth and gill development in the small-mouthed bass, Micropterus dolomieu Lacepede. John W. Price, Ohio State University. (Introduced by Raymond C. Osburn.) (Lantern; 10 min.)
161. Secondary sex characters in certain snakes. Frank N. Blanchard, University of Michigan. (Lantern; 10 min.)

By demonstration

162. Specimens of Urnatella gracilis from the Licking River, Kentucky. S. R. Williams, Miami University.

By title

163. The prenatal growth in weight and length of the digestive tube of the cat. Homer B. Latimer, University of Kansas.
164. The skeletal anatomy of Pliotrema, a six-gilled pristiophorid shark. L. Hussakof, American Museum of Natural History.
165. The structure and function of the facial pit in the pit vipers. W. Gardner Lynn, Johns Hopkins University. (Introduced by the Secretary of the Society.)
166. The heart of the lungless salamander. A. Grace Meekel, Cornell University. (Introduced by the Secretary of Section F.)
167. Hatching twenty-two-year-old eggs and the reduction of vigor in the rotifer Hydatina senta. David D. Whitney, University of Nebraska.
168. The retention of dye in intra-vitam methylene-blue preparations. Elbert C. Cole, Williams College.

ECOLOGY

Read

169. Ecology of certain oriental land fishes and crustaceans. A. S. Pearse, Duke University. (Lantern; 15 min.)
170. A study of fish from a small pond in a Sphagnum peat bog in Ohio. R. V. Bangham, College of Wooster. (5 min.)
171. Tagging of fish in Ohio. R. V. Bangham, College of Wooster. (10 min.)
172. The bottom-shore fauna of western Lake Erie: a population study to a depth of 6 feet. Frederick H. Krecker and L. Y. Lancaster, Ohio University and Kentucky State Teachers College. (Lantern; 12 min.)
173. Notes on food and winter habits of Triturus viridescens. Ann H. Morgan and Margaret Grierson, Mount Holyoke College. (Lantern; 8 min.)
174. Territory in mammal life. W. L. Strunk, University of Michigan. (15 min.)

By demonstration

175. The fauna of Grand Isle, Louisiana: Preliminary survey of the free-living fauna exclusive of Protozoa and mammals. Ellinore H. Behre, Louisiana State University.
176. The frogs of the United States and Canada. Albert Hazen Wright and Anna Allen Wright, Cornell University.
177. Methods for ecological studies on birds. S. Prentiss Baldwin and S. Charles Kendeigh, Baldwin Bird Research Laboratory, Cleveland. (Introduced by L. J. Cole.)

By title

178. The life cycle of the California oyster (Ostrea lurida). W. R. Coe, Yale University.

PARASITOLOGY

Read

179. Notes on the life history of a proteocephalid from Rana clamitans. Lyell J. Thomas, University of Illinois. (Lantern; 10 min.)
180. The excretory system as a method of classification of digenetic trematodes. Ernest Carroll Faust, Tulane University Medical School. (Lantern; 15 min.)
181. A comparative study of the viability of the ova of Diphyllobothrium latum from different hosts. H. E. Essex and T. B. Magath, Mayo Clinic. (Lantern; 10 min.)
182. Some studies on the changes in virulence of a strain of Trypanosoma equiperdum, using albino rats as hosts. T. C. Carter, Northwestern State Teachers College and University of Wisconsin. (Introduced by N. R. Stoll.) (15 min.)
183. The validity of Taenia confusa Ward, a tapeworm of man. Marlowe G. Anderson, Northwestern University. (Introduced by F. D. Barker.) (Lantern; 7 min.)
184. The biochemistry of the sheep tapeworm Moniezia expansa. Otto A. Tischer, Northwestern University. (Introduced by F. D. Barker.) (Lantern; 8 min.)
185. The effect of certain environmental factors on the development and hatching of the eggs of blood flukes. Angelo R. Onorato and Horace W. Stunkard, New York University. (7 min.)
186. The effect of dilution of sea-water on the activity and longevity of the cercariae of Cryptocotyle lingua. Horace W. Stunkard, New York University. (8 min.)
187. An addition to the list of acanthocephalan parasites of Amphibia. Benjamin P. Young, Cornell University. (Lantern; 7 min.)
188. Collyriclum faba as a parasite of poultry. William A. Riley, University of Minnesota.

By demonstration

189. Another trematode with two anal openings. Horace W. Stunkard, New York University.

By title

190. Vitamin requirements of intestinal nematodes. James E. Ackert, Kansas State Agricultural College and Molteno Institute, University of Cambridge, England.
191. The new larval nematodes from Rana sp. A. C. Walton, Knox College, Galesburg, Illinois.
192. An amphistome cercaria, Cercaria poconensis, with branched main excretory tubes. Charles H. Willey, New York University. (Introduced by the Secretary of the Society.)
193. The occurrence of Typhlocoelom flavum in North America. Charles H. Willey, New York University. (Introduced by the Secretary of the Society.)
194. The life history of Epibdella melleni MacCallum 1927, a monogenetic trematode parasitic on marine fishes. T. L. Jahn and L. R. Kuhn, New York University and The New York Aquarium. (Introduced by H. W. Stunkard.)
195. Specificity of infectious myxoma virus of rabbits. Roscoe R. Hyde and Raymond E. Gardner.

ABSTRACTS

An alphabetical author index for these abstracts will be found on pages 366–370.

COMPARATIVE AND GENERAL PHYSIOLOGY

Read

1. Locomotion and response in Difflugia. S. O. Mast, Johns Hopkins University. (8 min.)

The fleshy part of Difflugia is in structure much like Amoeba.

Locomotion is brought about by the extension of pseudopods, one after another, and attachment to the substratum at the tip, followed by contraction, which pulls the shell forward.

The elastic strength of the plasmagel is lowest at the tip of the pseudopod. This results in contraction of the plasmagel elsewhere and this forces the plasmasol out through the plasmagel tube, causing expansion at the tip of the pseudopod.

When the tip of an extended pseudopod comes in contact with the substratum, much of the plasmasol in it gelates. This is followed by marked increase in the thickness and in the elastic strength of this layer throughout the entire pseudopod, after which it contracts.

Difflugia leaves the shell if it is broken, and then moves about naked. At first the process of locomotion is the same as it was with the shell; then contraction in the pseudopods decreases until it disappears, after which locomotion is like that in Amoeba.

If a needle is brought in contact with the side of an extended pseudopod, the plasmagel in this region thickens greatly, then contracts. This results in rapid and sharp bending in this region.

The contraction of the pseudopod is due largely to increase in thickness and decrease in length of portions of the plasmagel after its formation by gelation of plasmasol. This is probably due to molecular polarization.

2. Response in Amoeba to localized photic stimulation. S. O. Mast, Johns Hopkins University. (7 min.)

If the light on the entire body of an active amoeba is rapidly increased, all streaming movement ceases. The same result is obtained if the increase in light is confined to the anterior end, but if all except the anterior end is illuminated, streaming does not cease.

If half of the anterior end is illuminated, streaming ceases in that half, but not in the other.

If one of the pseudopods in a multipodal amoeba is illuminated, streaming ceases in that pseudopod, but not in any of the others.

Light produces gelation of the plasmasol adjoining the plasmagel. This results in increase in the thickness and in the elastic strength of the part of the plasmagel illuminated. At the anterior end the plasmagel is relatively very thin and weak. It is therefore continuously stretched by pressure against it, owing to contraction of the plasmagel in other regions where its elastic strength is greater. When the plasmagel at the anterior end is illuminated, it increases in thickness, owing to gelation, until its elastic strength equals that in other regions; then pressure inward is equal in all directions and streaming stops.

Gelation produced by local illumination does not result in gelation elsewhere. There is nothing in the nature of an impulse which produces gelation at a distance, but the effect of local illumination is transmitted to all parts of the system in the form of change in pressure.

3. The rate of light adaptation in Eristalis tenax. William L. Dolley, Jr., University of Buffalo. (Lantern; 15 min.)

The rate of light adaptation in the eye of Eristalis depends upon the illumination. When a dark-adapted eye is exposed to an illumination of 12 meter candles, the eye becomes completely adapted to this illumination in about thirty minutes. When a dark-adapted eye is exposed to an illumination of 105 meter candles, the eye becomes completely adapted to this illumination also in about thirty minutes. Similarly, when a dark-adapted eye is exposed to an illumination of 257 meter candles, the eye becomes completely adapted to this illumination in about thirty minutes.

These results show that the rate of light adaptation depends upon the illumination. They seem to indicate that the rate varies directly with the illumination.

4. The activating influence of light upon certain aquatic arthropods. William C. Stehr, Ohio University. (Introduced by D. E. Minnich.) (Lantern; 5 min.)

In a previous report it was stated that there is a definite inverse relationship between the intensity of the stimulating light and the reaction time of the animal in all species investigated, namely: Asellus communis Say, Hyallela knickerbockeri Bate, Cambarus sp. (Crustacea), Belastoma flumineum (Say), Lethocerus americanus (Leidy), and Ranatra americana Montandon (Hemiptera). There is a latent period in the response of Lethocerus which is generally from 0.4 to 0.6 second in duration. The limit of stimulating efficiency of light for Lethocerus is reached between 13 and 25 m. c., which differs from the results obtained with other species. The process of dark adaptation in Lethocerus is regular and is complete in about fifty minutes. These results are very similar to measures of the same phenomenon in clams, Crustacea, and the conger eel. The results of the entire investigation are very similar to the results obtained by Hecht with the clam. However, formulae thus far developed in investigations of this sort do not express accurately the results obtained in these experiments. Inability to overcome the inhibitions and other influences of the central nervous system upon the responses is probably the greatest factor giving rise to differences between these arthropods and the forms used by other investigators in which the influence of the central nervous system was practically negligible.

5. The nature of the visual cells of lampreys. G. L. Walls, University of Michigan. (Introduced by A. F. Shull.) (Lantern; 15 min.)

For three-quarters of a century there has been a controversy as to the nature of the visual cells of lampreys. In these forms two kinds of cells are present; they may be designated as 'long' cells and 'short' cells. In all, three species have been investigated previous to the author's work.

Three authors have claimed that the two types of cells are not distinct from each other and that they are not sufficiently differentiated from ancestral cells to merit being termed 'rods,' 'cones,' or both. Four investigators, guided chiefly by the shape of the cells, have considered both types to be cones. Five other workers, guided chiefly by the disparity in size of the two types of cells, have designated the long cell as a rod and the short cell as a cone.

The author has studied the retinae of eight species of lampreys, and presents cytological, histological, embryological, and physiological evidence for still a fourth theory—that the long cell is a cone and the short cell a rod.

6. The effect of darkness and temperature on the excised eye of the frog. L. B. Arey and W. K. Jennings, Northwestern University Medical School. (Lantern; 10 min.)

Previous experimentation has shown that the characteristic positional changes of the retinal elements can be approximated when dark-adapted eyes of the frog are enucleated and placed in a suitable immersing solution in the light. The reverse experiment (light to darkness), however, has not been successful. It was thought that the normal peritoneal fluid might furnish a more favorable medium in which retinal movements could occur. To test this possibility, experiments were carried out by introducing enucleated eyes into the opened belly cavity of a frog and then sewing up the small incision. After such procedure, the previously light-adapted retina shows a variable retraction of pigment and elongation of the cones, as is characteristic in the normal animal under like conditions of darkness. When the effect of temperature was similarly tested, it was found that some thermal influence is operative. All these responses are independent of the nervous system and, apparently, of hormones. It would thus appear that the peritoneal fluid merely acts as an especially favorable medium in which reactions may proceed relatively unhampered.

7. The effects of x-rays on regeneration in Tubifex. R. G. Stone, University of Missouri. (Introduced by Winterton C. Curtis.) (Charts; 7 min.)

Posterior regeneration in Tubifex is inhibited after suitable irradiation with x-rays. This treatment destroys the neoblasts or reserve cells from which new mesodermal structures normally form, and there is no regeneration of these parts. The epidermis is likewise affected, as shown by the absence of mitoses, and epidermal regeneration does not occur on account of failure of this source of new material. The small knob formed at cut surface by irradiated worms arises by rearrangement of the terminal region of old body tissues. Previous investigators found that Tubifex replaces but three segments when anterior segments to the number of ten are removed. After thirty minutes' irradiation with x-rays, four to five anterior segments were removed from Tubifex and the

individuals checked against controls. Such irradiated worms fail to regenerate an anterior end. The cut surface is merely covered by an extension of the old epidermal cells. Later the body-wall muscles also extend across this area. No mitotic figures appear in any of the cells of this region, although the worms have been kept alive as long as fifty days without further change. Controls regenerate three new anterior segments within eighteen days. Material for formation of new structures arises by proliferation from epidermis and intestinal lining at wound surface, as shown by numerous mitoses in these layers. No neoblasts are present during anterior regeneration. This indicates that following this irradiation epidermal and endodermal cells do not divide and hence cannot furnish the new material as in the normal regeneration.

8. The effects of x-rays on regeneration in the nemertean, Lineus socialis. K. B. Coldwater, University of Missouri. (Introduced by Winterton C. Curtis.) (Charts; 7 min.)

Previous investigation has shown that the nemertean Lineus socialis exhibits great powers of regeneration. Small fragments of the body regenerate missing structures in fifteen to eighteen days. It has been further shown that the regenerating organs are formed from embryonic cells of the parenchyma and basal layers of the epithelium. These cells migrate to the cut ends, undergo rapid proliferation, and differentiate in the regenerating areas. Lineus socialis was irradiated with an x-ray exposure of 12,000 R. units and divided into fragments approximately 2 mm. in length. Irradiated fragments were checked against regenerating control pieces of similar size. Irradiated pieces healed the wounds, but failed to regenerate new organs. The pieces were otherwise normal in appearance and were killed forty-one days after treatment. Control specimens regenerated normally. Preliminary histological examination shows a lack of regenerated organs. Embryonic cells have been partially, if not completely, destroyed by the treatment. Other types of cells appear normal in sections. Regeneration is retarded, but not completely inhibited by lighter exposures to x-rays. Worms were exposed to 4000 R. units and cut into pieces for regeneration. A slight development of the brain and eyes was visible when they were killed at forty-one days after treatment.

9. Ultraviolet point radiation and the heart beat of Fundulus, frog, and turtle. M. A. Hinrichs, I. T. Genther, P. O. C. Johnson, and I. Milliken, University of Chicago. (Lantern; 8 min.)

The unscreened radiation from a Cooper-Hewitt quartz mercury-vapor arc was focused through a quartz rod drawn to a fine point, and placed at right angles to the horizontal axis of the burner. A bend of approximately 45° just back of the tip permitted microscopic study of the effects of radiation. The control of exposures was facilitated by mounting the rod on a universal joint and interposing a shutter between the arc and the end of the rod.

In each of the three vertebrate hearts studied, it was found that a local change in physiological activity, induced in the pace-making region, produced a change in the rate of heart beat. Short exposures accelerated and long exposures inhibited the rate of beat in each case.

Long exposures in the frog and turtle produced arhythmias and effects simulating heart block. It was possible, after a reasonal interval, to reestablish a normal rhythm in hearts of frog and turtle in which standstill had occurred. Following application of radiation at the sino-auricular node, the beat appeared first in the sinus region, then in the atrium, then in the ventricle.

10. Ultraviolet point radiation and the heart beat of Limulus. Marie A. Hinrichs and Ida T. Genther, University of Chicago. (Lantern; 7 min.)

Small animals were used, with average total body lengths of not more than 12.5 cm. The tubular heart was exposed by operation. (The control of the heart beat is neurogenic; the nerve cord runs along the median dorsal surface of the heart.) Ultraviolet point radiation as described above was used. The tip of the quartz rod was placed on various points along the cord; the locus and the length of exposure were determining factors in the effects obtained.

Pressure on the nerve cord due to too heavy contact of the rod had to be avoided, since it slowed the heart rate or even blocked conduction. In general, an increase in heart rate was obtained by short exposures in a given region, a decrease by longer exposures. Regional effects were established—an approximately equal rate increase was obtained along most of the cord; exposure just behind the joint, where the inhibitory fibers enter, slowed the rate considerably; exposure in front of the joint, where the stimulatory fibers enter, and exposure of the anterior cardiac ganglion increased the rate still further. Rapid recovery of normal rate followed exposures where induced variations were not marked.

Cutting the heart muscle did not eliminate the synchronism of the beat in the two parts of the heart, nor did it affect the conducting power of the nerve during exposure. No change in the heart rate resulted from exposure of the heart muscle itself or from exposure of the suspensory ligaments.

11. Pigment migration in the vermilion-spotted newt induced by ultraviolet radiation. H. H. Collins and E. A. Wolf, University of Pittsburgh. (Lantern; 8 min.)

The animals were subjected to ultraviolet radiation from a Hanovia lamp, at a distance of 17 inches, for twenty-minute periods three times per week. The most conspicuous pigmentary effect was observed in the migration of the red pigment which is very prominent in the color pattern of these animals. Ten days after the beginning of the experiments, the red pigment began to leave the spots where it normally occurs and was observed streaming dorsally toward the median line. As radiation is continued, the red pigment disappears entirely from the markings, leaving white spots where the red normally occurs, and concentrates along the dorsal median line. Much of the red pigment is lost in the abnormal molts induced by the treatment. The pigment migrates into the superficial portion of the epidermis and is cast off with the molted skin. No restoration of the normal pigment pattern has as yet been observed. There are marked individual differences in response to the treatment. Of the two subspecies, viridescens and dorsalis, the latter is much more susceptible.

12. Regeneration and metabolism experiments with electromagnetic fluxes. R. A. Muttkowski and Leo E. Buss, University of Detroit. (Lantern; 7 min.)

When treated with electromagnetic fluxes for daily periods of five minutes, cut Planaria regenerate in eleven days, the controls in fourteen days. Sixty and 80 volts are most effective; 120 volts retard regeneration, but the specimens are larger on completion of regeneration. In the metabolism experiments the CO_2 of treated and control bean and pea seeds was caught in baryta water and the amounts titrated. As 60 volts there is a 7 per cent increase in CO_2 production over the controls, at 80 volts virtually the same as the controls, and at 120 volts a 4 per cent less production.

13. On the reactions of oyster larvae to currents and to salinity gradients. T. C. Nelson and E. B. Perkins, Rutgers University. (Lantern; 15 min.)

Ten years' study of oyster larvae (O. virginica) in New Jersey coastal waters demonstrates that the distribution of the larvae is determined chiefly by the strength of the currents and by salinity gradients. Stratification of the water occurs in Barnegat Bay, resulting in surface salinities much below those which are found from middepth to the bottom, 2 to 3 meters. Transition from the overlying water of low salinity to those of high salinity beneath may occur within 1 decimeter, resulting in a sharp transition zone, the halicline. When the underbody of high-salinity water is not moving with the upper body of low-salinity water, a shearing plane occurs at the halicline, and immediately above this are found the oyster larvae in greatest abundance. At turn of the tide with slowing of the current the larvae occur in greatest abundance just above the halicline if present. If the water shows no distinct salinity stratification, the larvae descend to the bottom, to rise when the tidal current flows again. When the entire body of the bay moves as a unit from top to bottom, as in Delaware Bay and during rough weather in Barnegat Bay, the larvae occur in greatest numbers where the speed of the current is highest, regardless of salinity differences. Laboratory experiments show that oyster larvae tend to lie on the bottom in still water, rising when a current begins. Larvae are stimulated to swim by an increase in salinity, while a decrease in salinity is followed by a lessening of velar activity.

14. The sensitivity of the legs of the monarch butterfly, Anosia plexippus Linn., to saccharose and lactose. Almeda Anderson, University of Minnesota. (Introduced by D. E. Minnich.) (Lantern; 5 min.)

Using proboscis extension as an indication of chemoreception, the sensitivity of the legs of the Monarch butterfly, Anosia plexippus, to certain sugars has been tested. The methods used were similar to those of Minnich in work on Pyrameis atalanta. Animals hatched in the laboratory were kept on a water diet until death, trials being made with saccharose and lactose at regular intervals. On this diet they rarely responded to water and never responded to 64/100 M lactose. They never failed, however, to respond to 64/100 M saccharose. Determinations of the saccharose threshold were made for each animal, either daily or daily after the third day, with a series of concentrations ranging from 64/100 M to 1/409,600 M. There was considerable variation in the different individuals in the minimal concentration capable of eliciting consistent responses

during the last days of water diet. Of the fifty-four animals tested, fourteen, or 25 per cent, never consistently responded below 1/100 M. For nineteen, or 36 per cent, the minimal effective concentrations were between 1/100 M and 1/6400 M. For twenty-one, or 39 per cent, they were lower than 1/6400 M; six of these butterflies, or 11 per cent of the entire fifty-four, responded consistently to concentrations of 1/102,400 M or lower for at least one day. One exhibited this low threshold for three successive days. Since the upper limit of the threshold to saccharose of the human tongue lies between 1/64 M and 1/32 M, the legs of this butterfly may in some cases exceed the sensitivity of that organ 1600 times. No correlation was found between sex and sensitivity of the legs.

15. The contact chemoreceptors of the honeybee, Apis mellifera Linn. Dwight Elmer Minnich, University of Minnesota. (Lantern; 5 min.)

Using proboscis extension as an index of reception, the antennae and legs of the honeybee have been tested for contact chemoreception. Foraging bees returned to a food source were caught and mounted according to the technique devised by the author in his studies on flies. After mounting, the bees were fed sufficiently with 64/100 M saccharose to keep them alive for varying lengths of time, during which they were tested with 64/100 M saccharose and 64/100 M lactose, each trial being preceded by a check trial with water. Under appropriate nutritional conditions, a response was seldom effected by contact of either the antennae or the legs with water. 64/100 M lactose also failed to effect a response, except in rare instances. 64/100 M saccharose, however, effected a proboscis extension in the great majority of cases when brought in contact with the antenna or the first leg, but only rarely in the case of the third leg. (The second leg was not tried.) In the case of the antenna and the first leg many animals showed a 100 per cent response to such stimulation. Moreover, momentary contact with the distal end of the appendage often sufficed to bring about the reaction. These results show clearly that, in addition to the mouth parts, there are also important contact chemoreceptors concerned with food choice located in the distal ends of the antennae and the first pair of legs.

16. Group learning in goldfish. Carl Welty, The University of Chicago and Parsons College. (Introduced by W. C. Allee). (Lantern; 15 min.)

Goldfish, 45± mm. in length, arranged singly and in groups of twos, fours, and eights in aquaria, are conditioned to learn a simple maze by the reward method. Results from 102 fishes show that, on the average, the two larger groups run the maze in consistently and significantly less time than do the singles or groups of twos, and that the groups of twos perform in significantly less time than do the singles. For the first two trials the shortest time for performing the maze is found among individuals of the single class. The greater percentage of slow and refractory individuals is found among the singles. Results from 112 fish show that groups of four are conditioned to the maze in significantly less time either when conditioned individuals are added to each group or when unconditioned individuals are so placed in the aquarium as to act as lures. Indications are that groups plus conditioned fish learn more readily than do groups plus lures. There are indications that individual fish eat more in groups than when alone.

17. Weight and metabolism changes during the metamorphosis of the Japanese beetle (Popillia japonica Newman). Daniel Ludwig, New York University. (Introduced by the Secretary of the Society.) (Lantern; 10 min.)

Larvae of the Japanese beetle were collected in the field during February and March and placed at 25°C. Records of weight and metabolism changes were made at frequent intervals during metamorphosis.

The average weight of the male larvae increased to 226.5 mg. and of the female to 275.6 mg. During the prepupal stage there was a decrease in weight to 190.9 and 235.2 mg., respectively. There was a loss of 10 mg. during pupation and a slight loss of from 1 to 2 mg. during the pupal stage. The emergence of the adult male was accompanied by a loss of 66.2 and of the female of 76.1 mg. Determinations of the water content of late pupae and adults (recently emerged) showed that the weight loss on emergence is due entirely to loss of water and shedding of the pupal skin.

The rate of oxygen consumption per gram body weight showed the characteristic U-shaped curve. It began to decrease during the early prepupal stage and continued until the third day of pupal life. It then increased until the end of the pupal stage. The emergence of the adult was accompanied by a sharp increase in oxygen consumption. The respiratory quotient of the larvae fluctuated from 0.7 to 1.0 and in the pupae from 0.4 to 0.7. The emergence of the adult was accompanied by an increase in respiratory quotient.

18. The developing grasshopper egg. Eleanor H. Slifer, State University of Iowa. (Introduced by J. H. Bodine.) (Lantern; 15 min.)

Ordinarily, when kept at a high temperature (25°C.) from the day of laying, eggs of the grasshopper, Melanoplus differentialis, develop rapidly for about three weeks, at the end of which period the embryo extends nearly one-half the length of the egg. Growth then stops abruptly, and dividing cells, very numerous in twenty-day embryos, are almost entirely absent in those a week older. This quiescent condition persists for several months. Growth then recommences and hatching takes place about two weeks later. In rare instances hibernation fails to occur, a few of the eggs in a single pod—all of which were laid by one female—develop without a pause and hatch about the thirty-eighth day. This unusual behavior appears to be inherited. By selecting through several generations for this non-hibernating habit, a stock of Melanoplus differentialis has been secured in which the proportion of early-hatching grasshoppers is much higher than that previously found in nature—in one case 95.7 per cent of the eggs in a pod being of the desired type. By continued selection it is hoped that pure strains of hibernating and non-hibernating animals will be obtained which can be compared physiologically, cytologically, etc., in an endeavor to discover some of the causes for the sudden cessation of mitosis, in the former type, at the close of the third week of embryonic development; as well as its resumption after a prolonged period of inactivity.

19. Heart-beat reversal in a crane-fly. John H. Gerould, Dartmouth College. (Lantern; 10 min.)

Direction of heart beat in Pachyrhina ferruginea was easily observed after removing head, wings, and legs. This amputation had little immediate effect, so far as known, on the action of the dorsal vessel, clearly visible in abdominal segments 3 to 5. The alternating series (phases) of forward and backward beats were remarkably regular, averaging in the first set of observations 63 beats forward, 36.2 backward. There was a gradual, but very distinct, slackening in the rate of pulsation during each forward phase and acceleration during each backward phase. Ten beats in seven and one-half seconds at the beginning of forward phases diminished to ten in ten seconds at the end; backward beating, beginning with an average of ten in ten and one-half seconds, regularly increased to ten in eight seconds. Thus the metabolic rates at the two opposing centers from which waves arise were in this case evenly balanced, but in the same headless individual eighteen hours later a persistent backward phase of over 500 beats was observed.

20. The functions of the tracheal gills in larvae of the caddis fly, Macronema zebratum Hagen. Ann H. Morgan and Helen D. O'Neil, Mount Holyoke College. (Presented by Helen D. O'Neil; introduced by A. E. Adams.) (Lantern; 15 min.)

The larvae of Macronema zebratum live in swift-flowing water.

The natural conditions were imitated as nearly as possible during the months in which this experimental study of the function of the gills was being made. After several tests, it was determined that from a physiological point of view the tracheal system of the larvae is a closed one.

The whole equipment of fifty-eight tracheal gills was removed at one time without serious results to the larvae. The tracheal gills did not regenerate. The larvae lived without gills for eight months and finally pupated.

Amputation of gills reduced oxygen consumption only slightly, sometimes not at all. In oxygen intake gills are only accessory organs, oxygen intake occurring through the body integument.

Normal larvae eliminate carbon dioxide more rapidly than larvae without gills.

In the presence of excess carbon dioxide, larvae without tracheal gills consume less oxygen than normal ones, succumb to inactivity sooner, and recover more slowly. In carbon-dioxide elimination tracheal gills are important respiratory organs.

The rectal tracheal-blood gills play no significant part in the respiration.

21. Respiration of the puffer fish. F. G. Hall, Duke University. (Lantern; 12 min.)

A method is described whereby environmental factors which affect the respiration of fishes may be studied. The puffer fish, Spheroides maculatus (Bloch and Schneider), was used and experiments were conducted to show the influence of variations in temperature, carbon-dioxide tension on the quantity of water pumped over the gills, the respiratory rhythm, oxygen consumption, and the ability of the gills to remove dissolved oxygen from the water. Variations in

temperature and carbon-dioxide tension apparently had the most pronounced effect on respiration, while oxygen tension and hydrogen-ion concentration per se had much less. When fishes were subjected to temperature variations ranging between 12° and 22°C., the quantity of water pumped over the gills varied directly with the respiratory rhythm, but the quantity of oxygen removed was independent of the respiratory rhythm. In other words, it seems that dissolved oxygen passes into the blood through the gills at a maximum rate, without regard to certain variations in the physicochemical state of the surrounding medium.

22. A physiological study of gastric motility in the crab (Cancer productus). T. L. Patterson, Detroit College of Medicine and Surgery and The Hopkins Marine Station, Stanford University. (Lantern; 15 min.)

Comparative studies in the physiology of the gastric motor mechanism has been extended to the Crustacea, the large crab of the Pacific Coast being employed. The animals under experimentation were kept in a large vivarium provided with well-aerated, running sea-water. The chelae were wedged and the balloon introduced via mouth. Amputation of the endognathal palps arising from the meropodites of the third pair of maxillipeds is also desirable. The contractions of the gastric mill are periodic, the periods increasing in activity and length until in extreme fasting they become almost continuous on an elevated tonus. In the earlier stages of fasting there is a marked increase in the gastric tonus during the period of activity which terminates in a fall. The individual contractions of the gastric mill are rapid, with practically no intervening periods of rest, while the small and rapid oscillations seen in the curves are due to the action of the balers (terminal appendages of the exopodites) of the various pairs of mouth parts by whose vibratory motion water is kept flowing over the gills. Body movements or the introduction of small quantities of weak alkali or acid into the water near the animal produce temporary inhibition of the gastric contractions. Cutting the anterior pair of gastric muscles or isolation of the stomach from the cerebral ganglion (brain) by sectioning the circumesophageal commissures is without effect, thus demonstrating that the gastric activity in this animal is independent of the higher nervous centers.

23. The mechanism of deposition of calcium to form extraskeletal bone in the adult animal—a preliminary study. Clay Ray Murray and Margaret R. Murray, Columbia University. (Lantern; 15 min.)

This study is based on previously completed work in vivo dealing with new bone formation in the adult animal. The purpose of the study is to secure answers to the following questions: 1) Since the calcium utilized in fracture healing and extraskeletal bone does not come directly from the blood serum as needed, but is concentrated locally unprecipitated, what mechanism is responsible for its precipitation in the tissues to form bone? 2) Is specific cell activity involved in this mechanism? 3) How is the local concentration accomplished?

The experimental plan: action in tissue-culture medium of various concentrations of organic calcium at varying pH, with and without growing cells. This report embraces: A) Methods used in study. Hanging-drop cultures, with and without cells, were made of rat plasma and embryonic extract. To these

preparations were added varying amounts of calcium hexose monophosphate in solution, and calcium precipitation was studied in the plasma clot after the addition of NaOH N/100 or N/20, or of phosphatase extracted from young bones (Robison's method), or both. B) Results of preliminary study. 1) Without the presence of cells, the deposition of calcium can be made to occur in the exact pattern of new-forming bone by variations in calcium concentration and pH of the medium. 2) The introduction of a phosphatase from (dead) tissue extracts produces apparent intensification of the process observed in 1. 3) The growth of living cells, either fibroblastic or osteoblastic, produces no variation in the process observed in 1.

24. Histological changes in the maxillary-sinus mucosa following irrigations with calcium-precipitating substances. W. F. Wenner, Washington University. (Lantern; 15 min.)

The maxillary sinuses of rabbits and rats were irrigated with aqueous solutions of sodium alizarin sulphonate for ten days at forty-eight-hour intervals. Twenty-four hours after the last irrigation, histological examination of the sinus mucosa of both rats and rabbits showed marked infiltration of eosinophilic leucocytes into the basement membrane, with emigration of eosinophils into the lumen. Most of the cilia had disappeared from the columnar epithelium. Three months after the last irrigation, rats had chronic sinusitis. Histological sections of the sinus mucosa of these animals showed desquamation of epithelium, complete absence of cilia, marked infiltration of lymphocytes and eosinophils into the basement membrane and submucosa, with degeneration of mucous glands.

Sodium citrate and ammonium oxalate produced similar changes in the sinus mucosa of rabbits. With complete paralysis of the cilia, stagnation with subsequent infection results.

Ciliary action of small excised fragments of the lining epithelium of the maxillary sinus stopped when 0.3 per cent to 0.5 per cent solution of alizarin was added to the medium. Citrates and oxalates likewise brought about a cessation of ciliary movement.

Eosinophils appeared in the basement membrane following irrigations with physiological NaCl.

KCl produced a marked thickening of the basement membrane, lymphocytic infiltration and almost complete destruction of the epithelium.

$CaCl_2$ in isotonic and hypertonic solutions produced no noticeable histological change in the sinus mucosa.

25. The symbiosis between the wood-feeding roach, Cryptocercus punctulatus Scudder, and its intestinal flagellates. L. R. Cleveland, Harvard University Medical School. (Lantern, charts; 15 min.)

Every individual of the large, primitive, wingless, wood-feeding roach Cryptocercus punctulatus Scudder of the Appalachian mountains harbors in very great numbers several genera of undescribed hypermastigote and polymastigote protozoa. When deprived of its wood-digesting protozoa and fed partially decayed wood or cellulose, the roach dies within two to three weeks if not reinfected, but when fed completely decayed wood, it is able to live for two or three months.

This roach, like termites, is able to live on cellulose, but when fed other carbohydrates, its protozoa die, and their death is followed shortly by the death of the roach. The protozoa, then, are not only able to digest cellulose, but this appears to be the only carbohydrate which most of them can utilize.

The protozoa of this roach are closely related to termite protozoa and many of them are capable now of living in termites (Termopsis), but they resemble the protozoa of one termite no more than those of another and are no more closely related to the protozoa of termites from the Appalachian region than to those from other regions. They are probably the ancestors of termite protozoa. If so, the symbiosis between them and the roach has been established a very long time. It may be older than termites. When the protozoa of other roaches (particularly those of the subfamily Panesthinae, which is composed of twelve genera besides Cryptocercus) are studied, we will know whether termites got their extensive fauna of symbiotic protozoa from roaches or not.

26. The relations of numbers of animals to survival time in toxic concentrations of electrolytes. J. R. Fowler, University of Chicago and University of Louisiana. (Introduced by A. E. Emerson.) (Lantern; 15 min.)

An attempt is made to analyze the causal factors underlying differential survival between single individuals and groups of Daphnia when exposed to different concentrations of toxic solutions of various electrolytes. The conductivity method of measuring concentrations of electrolytes does not detect any change brought about by Daphnia living in the solutions.

When based on oxygen consumption, the metabolic rate of groups of Daphnia is markedly lower than that of single individuals in the same solution. This difference in metabolic rate between groups and single animals is not due primarily to lowered oxygen tension in the medium of the group. On the other hand, this reduced rate of metabolism of groups is largely due to the effects of accumulated carbon dioxide in the medium surrounding the group. This reduced rate of metabolism makes it possible for groups of animals to survive significantly longer than the relatively rapidly metabolizing single individuals when exposed to strong toxic solutions. Exactly opposite results are obtained with weaker concentrations.

This type of differential survival can be explained on the basis of Child's theory of differential susceptibility and differential acclimation for slowly and rapidly metabolizing animals when exposed to various injurious agents.

With hydroxide solutions carbon dioxide influences differential survival between groups and single individuals by reacting with the solutions and differentially reducing their concentrations.

27. A study of magnesium anesthesia. L. V. Heilbrunn, University of Pennsylvania. (Lantern; 8 min.)

In a recent monograph it was suggested that the activity of protoplasm is primarily dependent on a reaction or series of reactions that involve calcium. Reasoning from this standpoint, it was thought probable that magnesium anesthesia depends on a calcium antagonism. This is indeed in accord with older evidence. Magnesium anesthesia can conveniently be studied in sea-anemones.

Small anemones are reared in finger-bowls and furnish good experimental material. Anemones are anesthetized by the addition of varying amounts of an isotonic magnesium-chloride solution. The number of cubic centimeters of the solution necessary to produce a given degree of anesthesia is rather constant for a given anemone on any one day. Sea-water restores the irritability of the anemone. This recovery in sea-water is due to calcium ion, for no other ion in the sea-water is effective. The antagonism between magnesium and calcium can be studied quantitatively. Varying amounts of calcium ion are added to sea-water, and then the amount of magnesium ion necessary to produce anesthesia is determined. When one plots concentration of calcium against concentration of magnesium, peculiar curves are obtained. As the concentration of calcium rises, the concentration of magnesium necessary to produce anesthesia rises also. However, this rise is not gradual, but occurs in well-marked jumps. The amount of magnesium necessary to antagonize a given quantity of calcium is also a function of temperature. It is hoped that eventually the results will throw some light on the actual mechanism of the antagonism between magnesium and calcium.

28. The action of sodium, potassium, and calcium ions on the protoplasmic viscosity of Amoeba dubia. L. V. Heilbrunn and Kathryn Daugherty, University of Pennsylvania. (Lantern; 7 min.)

Earlier work showed that calcium chloride decreased protoplasmic viscosity of sea-urchin eggs and of Stentor, and that potassium and sodium ions had the opposite effect (Heilbrunn, '26). This result is in accord with various botanical observations. However, for the protoplasm of amoeba, it has been claimed (Chambers and Reznikoff, '26) that dilute solutions of calcium increase viscosity and that sodium and potassium solutions decrease protoplasmic viscosity. These results were based on impressions gained with the microdissection needle as well as on the fact that in sodium and potassium solutions the granules of the amoeba were observed to fall through the protoplasm.

Actual determinations of protoplasmic viscosity by the centrifuge method show that calcium decreases the protoplasmic viscosity of Amoeba dubia. Eleven determinations showed an average decrease of 42 per cent. In sodium-chloride solutions the granules were never observed to fall. In such solutions eleven determinations showed an average increase of protoplasmic viscosity of 40 per cent. When immersed in potassium-chloride solutions, the movement of the amoeba ceases, and owing to this stoppage of movement, the granules sink. If the action of gravity is prevented by rotation or agitation, the granules do not sink. It is then possible to make determinations of protoplasmic viscosity. These show that the viscosity in potassium-chloride solutions increases about 23 per cent (average of seven determinations).

It is therefore evident that calcium, potassium, and sodium ions act on amoeba protoplasm in the same way that they act on various other types of living substance.

29. Molecular organization of the protoplasm of amebas. A. A. Schaeffer, University of Kansas. (Lantern; 15 min.)

Since direct observation of discrete molecules is impossible, indirect methods must be employed to detect their presence and nature of organization, following

the methods of physical science. The evidence for protoplasmic organization may be classified as follows:

1. In the protoplasm of the ameba there exists a mechanism which makes it go in a spiral path around a glass rod with a preponderance of left turns.
2. The mechanism is submicroscopic.
3. Changes of light intensity have a marked effect on the ratio of right turns to left turns.
4. There are periods or nodes at regular intervals in the path of the ameba at which the direction of path changes.
5. The internodes have different lengths in different species of amebas.
6. The frequency of occurrence of the internodes of the various lengths (multiples of the unit length for each ameba) which occur in the paths of four species are represented by the exponential series:

$$\frac{1}{2^{\frac{y-1}{2}}}, \quad \frac{1}{2\,y-\frac{1}{2}}, \quad \frac{1}{3\,y-\frac{1}{3}}, \quad \frac{1}{4\,y-\frac{1}{4}},$$

where the base represents the length of the unit internode (section of rod circumference) and y the length of the section.

7. Disturbance of the left-right ratio is also correlated with general hypertrophy and, temporarily, with heavy feeding.

These data fall into a consistent system on the assumption that the underlying mechanism consists of right-turning and left-turning stereo-isomeric molecules which organize themselves into definite patterns, with attendant formation of fields.

30. Studies on immunity to rattlesnake venom (crotalin). J. Markowitz, H. E. Essex, and F. C. Mann, Mayo Foundation. (Lantern; 15 min.)

The many dramatic physiological responses following injections of rattlesnake venom into animals or its addition to perfused organs make this venom a unique antigen for use in investigating the physiology of immunity, since these responses can be employed as criteria of the immunity developed. Perfused non-immune rabbit hearts are rapidly incapacitated by small quantities of crotalin. The hearts of rabbits immunized to six lethal doses of crotalin react in an identical manner which suggests an absence of cellular immunity in the musculature. The marked swelling of the erythrocytes of the dog following addition of crotalin to blood in vivo and in vitro has been utilized in developing a method for titrating the immunity induced in dogs and rabbits. Immunity is measured in terms of the least venom sufficient to expand the erythrocytes 10 per cent. Immunity develops slowly in dogs being injected for the first time, and disappears in three to four months after injections are discontinued. Resumption of injections results in a rapid rise in the titer to as high as fifty times that of controls. Erythrocytes of immune dogs are not immune, but are protected by the antibodies in the plasma. Dogs with high titers show greater resistance to crotalin, as judged by blood pressure, than dogs with low titers. Immune dogs exsanguinated and transfused with non-immune plasma react to crotalin as do normal dogs. Simultaneous injections of horse serum and crotalin appear to retard the development of antibodies to the latter antigen.

31. The treatment of snake bite. Albert M. Reese, West Virginia University. (10 min.)

Potassium permanganate has long been considered an antidote for snake venom; but recently its efficacy for this purpose has been disputed by several workers connected with the Antivenin Institute of America, who claim to have proved the salt to be of little if any value.

Bruton, Noguchi, Weir Mitchell, and others claimed great potency for this remedy. Abel, Calmette, Hunt, and Stiles claim more or less decidedly that potassium permanganate is of distinct value in the treatment of bites by venomous serpents, especially if used promptly.

The writer is carrying on a series of experiments with rats to endeavor to prove what value, if any, the salt may have when injected promptly after the injection of the venom.

One of the chief difficulties in coming to proper conclusions from such experiments lies in the wide variation in the susceptibility of different individual animals to the same poison.

The minimum lethal dose per kilogram of body weight is many times greater in rats than in man.

32. Artificial insemination with motile sperm from ovariotomized fowl. L. V. Domm, University of Chicago. (Lantern; 8 min.)

Our recent studies have demonstrated the occurrence of spermatogenesis in ovariotomized fowl and explained conditions under which it arises. These cases were found on histological examination of serially sectioned gonads. This study revealed that only rarely does most of the gonad undergo spermatogenesis, but that almost invariably only a small part is involved. Continuous passage between spermatic tubules and vas deferens was not positively demonstrated.

The question whether spermatozoa from such cases are motile and capable of fertilization is interesting, but obviously could not be answered from studies on prepared material. Furthermore, since poulardes very rarely tread, special methods were necessary to determine this point. Surviving poulardes were subjected to special postmortem examination. Complete transverse incisions were made at close intervals throughout the gonad, and smears from each, also from contents of the vas, microscopically examined.

Twenty-eight poulardes were examined. No spermatozoa were found in vasa deferentia. Smears from each gonad excepting one were devoid of spermatozoa. This case (no. 1084) yielded motile spermatozoa in smears from transverse incision approximately 6 mm. from posterior end. The poularde ovariotomized when eight days old was killed three years and seventeen days later.

This part of the gonad was removed and mashed in approximately 6 cc. of Locke's solution. Equal parts of the suspension were introduced into the oviducts of three virgin laying hens. Eggs were collected for eighteen days and incubated. No fertility was found. A similar technique, previously developed, obtaining spermatozoa from the vasa deferentia of sexually active cocks yielded fertility.

33. Implantation of juvenile testicular tissue into the hypertrophied right gonad of ovariotomized fowl. L. V. Domm, University of Chicago. (Lantern; 7 min.)

In our early experiments sex inversion was incomplete because we failed to find spermatogenesis, though the individuals were evidently equipped to function as males. In order to determine whether these birds could function as such, special experiments were devised.

Sixteen poulardes showing well-developed masculine head furnishings were selected. Laparotomies were performed and into the right testis-like gonad of each juvenile testicular tissue was placed. Two short incisions were made on exposed surface of gonad approximately 8 mm. apart. With curved probe a tunnel was made between these and tissue introduced. The birds were kept under observation for approximately one year, then killed and examined.

Mating tests were negative. None were known to tread. External characterization was typically poularde. Cases showing successful implantations appeared no different from unsuccessful. Macroscopically, all but one of gonads appeared normal. In this case it was decidedly larger and irregular, indicating pronounced hypertrophy of engrafted tissues. Smears from three gonads showed numerous motile spermatozoa.

Histological examination revealed spermatogenesis in five cases. This was typically grafted testis tissue, principally composed of the following types of tubules. 1) In various stages of maturation without spermatozoa. 2) Similar, with spermatozoa. 3) Desquamating. 4) With no intact cells except a single layer of supporting epithelial cells which are similar to sterile tubules of host gonad. Evidence for spermatic infection of tubules of host gonad is difficult to determine. Even normal poulardes showing spermatogenesis are unable to function as males, owing to defects in urinogenital union and in behavior.

34. Determination and physiology of Bidder's organ. Emil Witschi, State University of Iowa. (Lantern; 15 min.)

Morphologically, Bidder's organ of the toad is a rudiment of gonadal cortex. Its compact structure is due to the rudimentary condition or the total absence of the medullar rete cords. Failure of the rete cords to reach the anterior part of the gonad primordium accounts for the female differentiation and the precocious development of the 'progonad.' Bidder's organ has the endocrine function of typical ovarial cortex.

35. Functional interrelations of anterior-hypophysial and gonad hormones. Carl R. Moore and Dorothy Price, University of Chicago. (Charts, lantern; 15 min.)

Oestrin injected into normal male rats produces injury to the testes and castrate conditions of seminal vesicles and prostate gland; also testis hormone injected into normal females suppresses oestrous cycles. This evidence, apparently supporting Steinach's antagonism hypothesis, demands other explanations, for we find also that male-female hormone mixtures stimulate normal reproductive accessories in castrated males and cornified vaginal smears in spayed females. Thus we find no evidence of chemical nor biological antagonism when hormones are introduced into gonadless animals.

The antagonism conception disappears on consideration of four well-substantiated postulates: 1) Gonad hormones act directly upon homologous, but are ineffective on heterologous, accessories; 2) gonad hormones have no direct effect upon homologous or heterologous gonads; 3) anterior-hypophysis hormone is required for gametogenetic or endocrine function of gonads; 4) gonad hormones suppress hypophysial function.

Oestrin, therefore, diminishes hypophysial output, producing consequently gametogenetic and endocrine hypofunction in normal males with resultant castrate accessories. Oestrin accompanied by fresh implants or hypophysial secretion produces no damage.

Testis hormone into females produces subeffective secretory states in the hypophysis, hence hypo-ovarian activity and cycle absence. Cycles are returned by introduced hypophysis secretion.

Testis-hormone-injected young males experience testis suppression similar to oestrin-injected males; consequently, the explanation cannot be hormone antagonism, but hypophysial depression from injected gonad hormone.

This conception also clarifies and correlates results obtained from vitamin-B-deficient and inanition diets, testis-hormone-injected hypophysectomized males, experimental cryptorchidism, and provoked precocious maturities. It enables one to visualize the dynamics of the normal oestrous cycle.

36. The gonad-stimulating and the luteinizing hormones of the anterior lobe of the hypophysis. H. L. Fevold, Frederick L. Hisaw, and S. L. Leonard, University of Wisconsin. (Introduced by M. F. Guyer.) (Lantern; 15 min.)

An aqueous pyridine extract of desiccated anterior lobe is extremely active for the stimulation of follicular growth and luteination of the follicles. By the injection of extract equal to 20 mg. of dried anterior lobe daily, immature rats (twenty to twenty-four days old) are brought to sexual maturity in three days, as evidenced by the opening of the vagina and the presence of follicular growth in the ovary. At the end of five days of continued injections, the entire ovary becomes a mass of corpora lutea and follicles. The increase in weight of the ovaries over controls varies between 800 and 1400 per cent.

This extract has been fractionated with the result that a water-soluble preparation has been obtained which promotes follicular growth with little or no luteination. Injections of the water-soluble fraction cause an increase in the weight of the ovaries over controls of 100 to 300 per cent. The water-insoluble fraction causes no increase in the weight of the ovaries of an immature rat. If the water-soluble is united to the water-insoluble fraction, we again obtain a luteinized ovary with an increase in the weight of the ovary corresponding to the original whole extract. The water-insoluble fraction also inhibits the cycle of a mature female, with production of large corpora lutea.

It would seem, therefore, that the water-soluble preparation contains a predominance of gonad-stimulating hormone, while the water-insoluble fraction contains the luteinizing hormone.

37. The function of follicular and corpus-luteum hormones in the production of a premenstrual endometrium in the uterus of castrate monkeys (Macacus rhesus). F. L. Hisaw, H. L. Fevold, and R. K. Meyer, University of Wisconsin. (Lantern; 15 min.)

The premenstrual endometrium is due to the combined action of follicular and corpus-luteum hormones. The follicular hormone promotes growth of the endometrium and development of the uterine glands, while the corpus-luteum hormone modifies these structures into the premenstrual type. The follicular hormone alone cannot produce a normal premenstrual endometrium, though bleeding after discontinuance of the treatment occurs during the degeneration of the structures built up.

A premenstrual endometrium can be developed in a castrate animal by first producing an oestrous condition in the uterus by administering follicular hormone followed by corpus-luteum hormone (corporin). If follicular hormone is given in large amounts at the same time corpus-luteum extracts are being injected, the effect of the corpus-luteum hormone is inhibited and the endometrium remains in the oestrous condition. After a thick endometrium is formed by injecting large amounts of follicular hormone (40 R. U. per day for thirty days), bleeding can be precipitated by lowering the dosage. The corpus-luteum hormone does not seem to postpone bleeding after the discontinuance of follicular hormone. The follicular hormone promotes growth of the endometrium and is associated with mitotic activity, while the corpus-luteum hormone does not seem to stimulate cell division, but modifies the structures resulting from follicular activity.

38. The potency of the anterior-pituitary sex hormone of normal and unilateral-castrated albino rats. Frederick E. Emery, University of Buffalo. (Introduced by the Secretary of the Society). (Lantern; 10 min.)

If one ovary is removed from a rat, the remaining ovary becomes enlarged and the physiological activity, as represented by the cornified cells in the vaginal smears, is increased. The cause of this hypertrophy is known to be related to the pituitary gland. A study has been made of the potency of the gonad-stimulating hormone of this gland taken from normal male and female rats at six months of age, and from litter mates which were previously unilaterally castrated. Two pituitary glands were grafted intramuscularly in female rats on the twenty-fifth and twenty-sixth days of age. These young recipients, each receiving a total of four pituitary grafts, were killed when thirty days old and the ovaries and uterus weighed. The results show that the potency of the anterior-pituitary sex hormone was not changed by unilateral castration in either male or female rats.

39. Ovariectomy and corpus-luteum-extract experiments in pregnant rats. George E. Johnson and Joanna S. Challans, Kansas State Agricultural College. (10 min.)

Bilateral ovariectomy in eleven rats six to twenty-one days pregnant resulted in abortion usually within forty-eight hours. Of three animals operated at nineteen days, one went to full term with mammary glands not functional. At twenty days, ovariectomy in four animals resulted in a large proportion of stillbirths

and, in one, functional mammary glands. At twenty-one days, operation resulted in some stillbirths in one of two litters.

Unilateral ovariectomy in eight rats ten to twenty-one days pregnant had no effects upon the pregnancy.

Bilateral ovariectomy followed by daily injections (0.15 to 0.3 cc.) of a corpus-luteum extract (method of Corner and Allen and a modification by Harris and Pfiffner, both giving same results) on thirty-two rats produced slight uterine development following operation at one to four days in five out of seven animals. At eight to seventeen days, bleeding resulted at 2.75 days average, indicating resorption, and abortion occurred in six of nine animals in 3.6 days average, and complete resorption in three animals. Of three rats spayed at eighteen days, two went to full term, with mammary glands functioning in one. Three rats operated at nineteen days continued to full term with viable young and functional milk glands. Daily injection of 0.4 cc. extract in a rat from ovariectomy at thirteen days of pregnancy to twenty-two days resulted in five viable young at twenty-four days with mammary glands functional.

Similar work on ground squirrels indicated greater potency of the extract, suggesting a species difference in response.

40. The reaction of chicks and immature fowls to the sex hormones. Mary Juhn and R. G. Gustavson, University of Chicago and University of Denver. (Introduced by Frank R. Lillie.) (Lantern; 15 min.)

A large flock of one-day chicks was reared in the laboratory under identical conditions. From this flock a group was selected and injected daily from one day to three weeks of age, the individuals then autopsied. The same procedure was repeated with chicks three weeks to six weeks of age, and so on to maturity. The hormone dosages, determined by previous experience with older birds, were kept constant for body weight. Within each group females were injected with the female and with the male hormones and males with the female and the male hormones. In every instance feathers were plucked previous to beginning injections and records kept of the regenerating plumage.

The female hormone caused growth and histological development of the oviduct in the female chicks of all stages. The male hormone caused comb growth in chicks of both sexes at all stages.

The male hormone caused no plumage modifications at any stage in either sex.

The female hormone provoked no changes in the feathering of the females; in the male, the regenerating feathering became modified in the female direction in the stage when the normal females were passing from the first neutral chick plumage to the juvenile female plumage. In the subsequent stages the regenerating plumage of the male exhibited successive conditions of the normal female controls of the same age.

The plumage thus differs from the remaining sex characters studied, inasmuch as precocious maturity may not be induced, but only successive strictly determined potentialities realized.

41. Integumental grafting as a means of analyzing the factors determining the secondary sexual characters of the domestic fowl. A. W. Kozelka, University of Chicago. (Introduced by Sewall Wright.) (Lantern; 15 min.)

The experimental work embodied in the investigation was confined to the transplantation of the comb, the wattles, and the spurs between infantile chicks. Male combs were grafted onto females and, for comparison, female combs were grafted upon individuals of the same sex. In like manner female combs were transplanted onto males, this being controlled by grafting male combs onto male recipients. The same procedure was followed in the case of the wattles and the spurs.

Conclusions are based on a total of 165 homoioplastic grafts in the adult stage, forty of which involved the transplantation of combs, twenty-one of wattles, and 104 of spurs.

Either male or female combs on male hosts averaged larger than corresponding grafts on female hosts. Similarly, wattles on male hosts, irrespective of the sex of the donor, were larger than similar grafts on female hosts. These facts would seem to indicate that the ultimate size of either the comb or the wattles is determined by the host rather than the donor.

Spur grafts from male donor developed into characteristically male spurs on either male or female hosts. The variation in size was approximately the same for each sex host. On the other hand, the female spurs on male hosts varied greatly, but for the most part approximated that of a female. Female spurs on female hosts retained a characteristically female character. Thus, the donor rather than the host appears to determine the size. It is not yet certain whether this result should bear a genetical or a physiological interpretation.

42. Temperature and male production in the cladoceran Moina macrocopa. L. A. Brown, George Washington University, and Arthur M. Banta, Brown University and Carnegie Institution of Washington. (Lantern; 15 min.)

An analysis of male production as controlled by temperature shows that the proportion of males produced by different degrees of crowding of the mothers is characteristic for each of several temperatures. From 14° to 21°C. male percentage increases with crowding, but temperatures immediately above and below this range show a maximum male production at intermediate degrees of crowding, greater crowding reducing the male percentage. A further increase in temperature tends to restore the direct relation between male production and the number of mothers reared together. Since temperature exercises this type of control over male production, an analysis of the temperature effect itself necessitates a grouping of the data obtained at a single temperature regardless of the degree of crowding. For this purpose all experiments involving from one to twelve mothers are used; the inclusion of more heavily crowded bottles would only serve to exaggerate a differential already evident. Such a tabulation gives the following results: 12° = 12.6%, 14° = 24.9%, 17° = 22.5%, 21° = 10.0%, 24° = 5.2%, 27° = 4.6%, and 30° = 20.2% males. Attention is directed to the similarity of the above effect to that obtained by Plough for crossing over in Drosophila, and the general similarity of both our data and Plough's to the curve for viscosity of protoplasm in relation to temperature as given by Heilbrunn.

43. Induced hyperthyroidism and sterility in rats. Bert Cunningham and C. W. Hooker, Duke University. (5 min.)

Studies were made on both male and female rats to determine the relation of the thyroid activity to sterility. Comparatively, the male seems more sensitive to the hyperthyroid condition than the female.

Female rats were fed 10 mg. of desiccated thyroid (Lilly) daily, after having been checked for fertility. The first series of experiments begun two years ago showed a decided reduction in reproductive rate, but sterility did not intervene early in a majority of cases. In the current experiments conducted with litter mates as control, one female died before first litter, four produced two litters after thyroid feeding was begun, one produced a single litter before death, while still another has produced a single litter. Omitting the first or 'check litters,' these compare favorably with the controls. There is a decidedly high death rate among the young, not so much due to the inherent weakness of the young, but to the increased cannibalistic attitude of the mother.

Male rats received 10 mg. desiccated thyroid daily and became sterile in variable periods of time, but usually well within thirty days.

When both male and female rats received 20 mg. daily, no litters were born after feeding was begun.

There is some evidence to show that the sterility in the male may be due to an increased temperature produced through the metabolic activation rather than through direct hormonal effect.

44. The hormone of the suprarenal cortex. W. W. Swingle and J. J. Pfiffner, Princeton University and Cold Spring Harbor, Long Island. (Lantern; 15 min.)

The suprarenal cortex of mammals contains a hormone necessary for the maintenance of life. This hormone can be extracted from the cortex by suitable agents and obtained in relatively pure form, free from proteins, lipids, and other contaminating substances. The extract is suitable for intravenous use and is non-toxic in large doses.

1. Administration of the cortical hormone to bilaterally adrenalectomized cats prolongs the life span indefinitely. The animals remain in normal condition, gain weight, exhibit normal sex behavior, and cannot be distinguished from control unoperated cats. Such treated animals succumb with typical symptoms of adrenal insufficiency following withdrawal of the extract treatment.

2. Prostrate cats, on the verge of death from adrenal insufficiency, can be restored to normal within forty-eight to seventy-two hours by injecting small amounts of the extract. After return to normal condition, the cats can be kept so for any length of time desired by a daily subcutaneous injection of small amounts of the extract.

3. The extract has been used successfully in the crises of Addison's disease. Intravenous injections revive prostrate individuals and restore them to normal activity within thirty-six to seventy-two hours.

45. The influence of ferrous iodide and linoleic acid on rats depleted of vitamin A. F. E. Chidester, A. G. Eaton, and N. K. Speicher, West Virginia University. (Lantern; 10 min.)

Seventy rats were placed in six different lots in an attempt to determine the influence of linoleic acid alone and in combination with the optimum doeses of ferrous iodide previously shown by us to cure xerophthalmia and inflammations accompanying vitamin-A deficiency.

The linoleic acid protected somewhat against vitamin-D deficiency, but was devoid of vitamin A. Yet, in combination with ferrous iodide, it induced growth and the ferrous iodide cured the xerophthalmia.

The experiments, begun June 6, 1930, indicate at the present time, November 12th, that linoleic acid, ferrous iodide, and irradiation of the diet or the animals not only restore the vitamin D, but partially replace vitamin A.

The rôle of unsaturated fatty acids, furnished rats on deficiency diets relatively rich in minerals but poor in fats, must be considered in any interpretation of the results which purport to indicate substitution for vitamins. Many of the papers that have already appeared in the literature are based on too few animals, and conclusions have been drawn at the end of a month or six weeks. Our results show that a temporary stimulation may indicate actual substitution for vitamins, but that in reality, the animals may have synthesized vitamins from stored foods. There are apparently a number of catalyzers effective in the production of vitamin A in depleted animals.

46. Body size as a factor in the metamorphosis of frogs. Edward F. Adolph, University of Rochester. (Lantern; 12 min.)

Tadpoles of Rana sylvatica and pipiens that were crowded in small aquaria were retarded in growth. At the time when metamorphosis occurred in uncrowded individuals, the crowded ones that had grown most were able to metamorphose at somewhat smaller sizes, becoming undersized frogs. But others were able to metamorphose only after prolongation of their larval existences, at various times throughout a whole year.

The maximum weights attained before metamorphosis by tadpoles unimpeded in their growth were, for certain broods kept at 19°C., 1230 mg. and 3400 mg. These normal individuals attained fore legs at fifty-four days and at 116 days after fertilization of the eggs from which they grew. The maximum sizes attained by certain tadpoles, of the same broods, that did not metamorphose within one month of the control ages, were 600 mg. and 2100 mg., respectively. A few individuals of the same weights were able to metamorphose before twice the normal ages. Evidently within limits a deficit in body size can be compensated by an increase of age; and it is found empirically that, for a certain brood $(W - d)\ (A - e) =$ a constant, where A is the age in days at which the fore legs appeared, W is the body weight after the completion of metamorphosis, and d and e are constants. No increase of size would allow metamorphosis to occur before e days, and neoteny would last indefinitely if sufficient body size to result in a frog of weight d were not attained. Body size is therefore a chief and a primary quantitative factor in the production of normal metamorphosis.

By demonstration

47. The surface precipitation reaction in the egg of Chaetopterus. D. P. Costello, University of Pennsylvania. (Introduced by L. V. Heilbrunn.)

When Chaetopterus eggs are crushed, the surface membrane ruptures and the protoplasm flows out. If a slow, even pressure is exerted, the exuding protoplasm normally becomes surrounded with a thin but visible surface film. This film is comparable to that produced by the surface precipitation reaction in Arbacia. In sea-water freed of calcium by oxalating or after washing in isotonic sodium chloride, the reaction does not occur. Strontium may be substituted for calcium in the reaction. Magnesium is ineffective. Sea-water at a pH of 3.0 or at a pH of 9.6 had no inhibiting effect on the reaction. The formation of bubble-like films under the raised vitelline membranes of cytolyzed eggs, which may be interpreted as an internal surface precipitation reaction, was observed with the following cytolytic agents; distilled water, ½ per cent saponin, 4 per cent amyl alcohol, 4 per cent ether, ½ per cent chloroform, 0.5 M magnesium sulphate, 0.3 M calcium chloride.

In eggs centrifuged to displace all the yellow pigment granules and then crushed, surface precipitation membranes were produced at the pole containing the heavy yellow granules, at the pole with the gray cap of fat globules, and at any intermediate point of the hyaline or granular zones. This would indicate that the pigment granules are not directly involved in the reaction, as they are in Arbacia.

No ovothrombin could be demonstrated in an extract of Chaetopterus eggs broken up in the presence of calcium.

48. The action of ultraviolet rays on Paramecium protoplasm. R. A. Troisi, University of Pennsylvania. (Introduced by L. V. Heilbrunn.)

When paramecia are exposed to ultraviolet rays, they undergo vacuolization. Numerous vacuoles were observed to form within the space of a few minutes. If, however, the paramecia are treated with oxalate solutions to remove calcium, much longer exposures fail to produce the characteristic vacuoles. The ultraviolet rays also cause a breakdown of the cell membrane or pellicle. This effect occurs independently of the presence of calcium.

49. Muscular differentiation in oysters exposed for diverse periods of time. Hoyt S. Hopkins, New York University.

Attached oysters were collected from a sea-wall near the Bureau of Fisheries Station at Beaufort, North Carolina. Those which had been living near the mean high-tide mark displayed a greater development of the white (tonus) fibers of the adductor muscle in relation to the gray fibers (closing muscle), both in mass and in cross-sectional area, as compared with oysters near low-tide mark. The ratio by weight of gray to white tissue in the muscles of eighteen oysters living at the upper limit of growth was 1.26 ± 0.03. In seventeen oysters taken from a level 2½ feet lower, where they were submerged during about three-fourths of the time, the ratio was 2.51 ± 0.09. Owing to the irregular aspect of the inner surfaces of the shells, the cross-sectional patterns of the muscles were diverse, and hence the relative size of the gray and white muscle portions may be influenced.

50. Rate of growth and body size in relation to the metamorphosis of frogs. Edward F. Adolph, University of Rochester.

An exhibit of charts showing changes of body weight in tadpoles growing under various conditions of crowding, and of preserved specimens of frogs newly metamorphosed at diverse sizes.

51. A biological method of determining circulation time. Theodore Koppányi, Georgetown University School of Medicine.

The complete circulation time in mammals is determined by injecting 0.25 mg. of epinephrine hydrochloride into the left common carotid artery. The injection is followed within one second by maximal dilatation of the left pupil, and, from six to nine seconds later, by dilatation of the right pupil. The time interval between the pupillary responses in the two eyes represents complete circulation time. The blood carrying epinephrine crossed both the pulmonary and a systemic circuit. That the results obtained are not due to the passage of epinephrine to the right eye directly by anastomoses in the carotid system was established by injecting methylene-blue solutions into the left common carotid artery, the blue coloring being noticed in the right common carotid artery below the level of injection of the dye on the left side in from six to seven and one-half seconds following injection.

52. A demonstration of blood-pressure apparatus. Edgar Altenburg, Rice Institute.

53. A demonstration of a working model of the compound eye. Edgar Altenburg, Rice Institute.

By title

54. Effects of white, green, and red lights of equal luminous intensity on the testis activity of the European starling (Sturnus vulgaris). Thomas Hume Bissonnette, Trinity College.

Commencing January 28th, for equal periods of six to seven hours nightly, 112 birds in cages of sixteen each were subjected to: 1) Total light from white incandescent bulbs of 131 lumens at 24 inches' distance; 2) similar light filtered through Corning glass, sextant green unpolished, of 117.25 lumens at 22.7 inches; 3) similar light filtered through Nonex H. R. pyrometer red glass, of 114.8 lumens at 22.5 inches; 4) controls without added light.

Till March 5th, this was added to normally lengthening daylight periods inside, thereafter to constant day length of nine and three-quarter hours, involving one and three-quarter hours' decrease in daylight. Light for groups 1, 2, and 3 was passed through window glass transmitting little ultraviolet.

Samples from all cages were killed and their testes studied at 19, 33, 49, 60, 74, 90, 100, 109 days. A second lot of fifty-four, similarly run from March 18th, were killed at 11, 25, 41, 51, and 60 days. These had daylight reduced about two and one-half hours and seven hours of added artificial light as above described.

Results show red light is most effective stimulant in later stages or longer time, white in earlier, and green least at all stages and perhaps inhibitory.

Red is most effective in preventing regression when daylight is reduced, white next, and green last.

Violet light of 0.124 as great luminous intensity as the above failed to stimulate spermatogenic activity, sometimes even inhibited it.

55. Effect of white light of different intensities upon the testis activities of the European starling (Sturnus vulgaris). Thomas Hume Bissonnette, Trinity College.

Groups of sixteen birds caged in a basement room, lighted by three large windows by day, were subjected to additional light after sundown from incandescent bulbs of 10, 15, 25, 40, 50 watts, and 60 watts with and without forced work, for equal periods, rising by fifteen to twenty minutes per night to six hours nightly, then kept constant, from November 9th to January 27th. Controls received no added light. Birds from each group were killed, December 13th, 23rd, and January 27th, and their testes studied.

Lights of all these intensities induce progressive testis changes. All birds, except controls, reach complete spermatogenesis in forty-four days, and some birds, from the 10- to 60-watt-treated cages, pass the climax of spermatogenesis before seventy-nine days, under increasing day length after December 21st, and regress to various degrees. Others do not.

Rate of acceleration of germ-cell activity increases with light intensity from 10- to 25-watt bulbs, possibly to 40; but above that, no constant differences resulted. Fifty-watt bulbs may be slightly more effective than 60-watt ones.

Added exercise caused lag of onset of light-induced changes, either progressive or regressive. Data suggest a refractory period before light-change effects appear, prolonged by added exercise, and idiosyncrasies of susceptibility to regressions.

Juvenile birds give neither so rapid nor so great increases in testis size and activity as mature birds under like conditions.

Total testis size produced in midwinter by electric light sometimes surpasses the spring normal.

56. Hydroid pigments. Nellie M. Payne, Marine Biological Association, Plymouth, England. (Introduced by the Secretary of the Society.)

The yellow and brown colors of the Sertularidae belong to the group of water-soluble pigments, known as flavones. The brown pigments of Thuiaria articulata and Serrularia plumila are oxidized products of yellow dyestuffs. The yellow colors of Sertularella gayi and S. polyzonias appear to be due to identical pigments. The white hydroid, Sertularia argentea, also contains flavones which can be demonstrated by dipping the zooids in NaOH or by making an aqueous extract alkaline (pH 8).

The yellow, orange, and brown colors in the Haleciidae and Plumularidae are insoluble in water, but readily soluble in ether, alcohol, CS, CCl, petroleum ether, acetone, or pyridine. Etherial solutions made from Antennularia antennina and A. ramosa yielded yellow rhomboid crystals with a greenish cast. The pigments obtained from Antennularia antinina, A. ramosa, Agophenia pluma, A. tubilifera, and Halecium halecinum belong to the caratinoid group. In general, these pigments are more soluble in CS than in other fat-solvents. Spectroscopic absorption bands obtained from various solutions of different hydroid species showed neither the typical absorption maps of carotin nor of xanthophyll.

57. Acclimatization to low temperatures in certain marine hydroids. Nellie M. Payne, Marine Biological Association, Plymouth, England. (Introduced by the Secretary of the Society.)

Mean thermal death-points of zooids of Sertularia plumila, Clava squamata, Sertularella gayi, and Sertularella polyzonias determined, first, by sudden immersion in a supercooled solution, and, secondly, by subjecting them to solutions cooled 1°C. a minute were not significantly different. By the first method they were: for Clava squamata, 20 ± 4.1; for Sertularella gayi, 15 ± 3.1; for S. polyzonias, 15 ± 2.3; for Sertularia plumila, 22 ± 3.3. When temperatures were lowered at the rate of 1°C. per hour the death-point for C. squamata was 24 ± 2.7; for Sertularia plumila, 25 ± 5.4. For S. gayi and S. polyzonias the thermal death-points obtained by this method were not significantly different from those obtained by previous procedures. Thermal death-points of these hydroids after being held for a week at —10°C. were: Clava squamata, 30 ± 2.9; Sertularia plumila, 27 ± 3.1; Sertularella gayi, 25 ± 3.7; Sertularella polyzonias, 28 ± 7.8. Thus in these species there was a distinct acclimatization. On the other hand, Hydractinia could be kept at 0°C. for two months, but lost cold hardiness to the intensity factor of heat, the loss being proportional to the length of exposure to zero. Mean thermal death-points of Hydractinia were: fresh, 15 ± 2.6; after one week at zero, 7 ± 1; after two months, 3 ± 0.8.

58. Oxygen concentration and oxygen consumption in solutions of different osmotic pressure. J. William Buchanan, Northwestern University.

Under the experimental conditions employed, the rate of oxygen consumption of Planaria in natural water is not changed by increasing the oxygen concentration of the water from 3.53 cc. to 13.84 cc. per liter. Hyman's results are confirmed. In distilled water the rate of oxygen consumption is constant, but distinctly subnormal between oxygen concentrations of 2.65 cc. and 5.35 cc. per liter. As the oxygen concentration is increased above this point up to 15.09 cc. per liter, the rate of oxygen consumption also increases. In Ringer's solution the rate of oxygen consumption is strictly dependent on the oxygen concentration of the solution from 3.79 cc. to approximately 14 cc. per liter. As the oxygen concentration is increased above this, the effect on rate of oxygen utilization becomes less; above 22 cc. per liter a decrease in rate is recorded.

Planaria dorotocephala contain by weight approximately 79 per cent water. In distilled water, as employed here, the water content increases about 5 per cent. In Ringer the water content is decreased about 19 per cent. The facts indicate that two conditions may be involved in the regulation of the rate of oxygen utilization in relation to oxygen tension in natural water: concentration of water within the organism, thus controlling the concentration of the oxidative enzymes; boundary regulation of the admission of oxygen. In this form, size of the animal and degree of development of its circulatory and respiratory mechanisms do not appear to be important.

59. Stimulation in Balanus and Sagartia by normal primary aliphatic aldehydes and acids. William H. Cole, Rutgers University.

For Balanus balanoides the threshold stimulating concentrations of the first four acids was practically the same. For Sagartia luciae also the first four acids showed equal stimulating efficiency. The concentrations of successive acids, however, decreased in the ratio of 1.5, up to and including pelargonic. Stimulation by these acids was not due to the hydrogen ion, since mineral acids at the same pH caused no response. Stimulation by the aldehydes in S. luciae and in Rana pipiens indicated a 3-to-1 ratio for successive concentrations from formaldehyde to valeraldehyde, inclusive, as was previously found for the normal primary aliphatic alcohols in S. luciae, R. pipiens, Planaria dorotocephala, and B. balanoides. The results indicate that the CH_2 group in the aldehyde molecule determines stimulating efficiency as was found for the alcohols, but that in the acids some additional factor (not the hydrogen ion) must also play a rôle.

60. Physiological significance of H-HS for the development of sea-urchin eggs. T. P. Sun, State University of Iowa. (Introduced by J. H. Bodine.)

The development of sea-urchin eggs in sea-water to which H-HS has been added was found to be retarded, as indicated by the number of cells in the circumference of the blastula. The rate of retardation seems to run parallel to the concentration of H-HS used. A concentration of 6^{-4} at pH 6.2 inhibits the development of the egg completely, while that of below 3^{-10} at the same pH value of sea-water has no effect. This action may probably be attributed to the H ion of H-HS which lowers the pH of the solution and so inhibits the cell division of the egg.

61. Inhibition of regeneration in two-headed planarians. E. D. Goldsmith, Harvard University and New York University. (Introduced by H. W. Rand.)

Double-headed forms of Planaria maculata in which the two heads are equal in size are produced by splitting typical individuals in the midline. Heads of unequal size are produced by splitting at one side of the midline. By varying the length of the incision, the extent of duplication of the region posterior to the heads can be controlled.

When one of the heads with varying portions of the duplicated region posterior to it is removed, regeneration of a head is in most cases retarded or inhibited. It was shown that this retardation or inhibition is not due to lack of head potency in the operated region, but is due to the presence of the remaining head. It was further demonstrated that the degree of inhibition depends on the relationships of the following factors:

1. Length of stump (the length of duplicated region left projecting beyond the common posterior part of the body).
2. Body level (level on anteroposterior axis at which the head is removed).
3. Distance (length of remaining head and duplicated region posterior to it, plus the length of stump).
4. Size and activity of heads.

The work is being continued.

62. Production of supernumerary heads by radial incisions in Planaria maculata and Phagocata gracilis. E. D. Goldsmith, Harvard University. (Introduced by H. W. Rand.)

Planarians with a supernumerary head in the middorsal region have been observed in stock cultures. Similar forms have been produced experimentally.

With a cataract knife, incisions approximately 1 mm. in length, radiating from a center, were made in individuals of Planaria maculata and Phagocata gracilis. The incisions were made 1 to 3 mm. posterior to the eyes, in animals ranging from 9 to 11 mm. in length. The operated region bulged and gave rise to an outgrowth, which in a few cases developed into a typical head. The original head was not removed.

The results obtained in the present work are similar to those obtained by Child (in Corymorpha) and by Santos (head transplants in Planaria maculata and Planaria dorotocephala).

Further experiments are being conducted to determine to what extent various regions of the body are capable of head formation under the influence of similar stimuli.

63. A study of the innervation of the heart of fish embryos. Floyd J. Brinley, North Dakota State College.

Unpublished experiments on the effect of caffeine on the embryonic chick hearts indicate that the rate of the auricular beat of the prevagus heart is not altered after treatment with caffeine, but the ventricular contractions become slow and irregular. In the postvagus, after the addition of caffeine, the auricular as well as the ventricular beat becomes slow and irregular. This indicates that caffeine affects the cardiac rhythm mainly through its action on the nerves. In the same manner the time of innervation of the hearts of fish embryos may be studied.

Eggs of yellow perch (Perca flavescens), log perch (Percina caprodes), and a shiner (Notropis delicious) were treated with caffeine at various intervals from the times the hearts started to beat until the eggs hatched. Caffeine produced no effect on the rate of the heart beat of yellow perch until sixty hours after the heart started to pulsate. After that time, the beat becomes irregular and the rate much slower. No effect was noticed on Percina until eighty-five hours after fertilization, or thirty hours after the heart started to beat. Cardiac contractions in Notropis started about forty-five hours after fertilization; caffeine affects the heart almost immediately after pulsations start.

The break in the rate curve seems to indicate the time the vagus innervates the heart. The time depends upon the rapidity of development of the embryos. Eggs of the perch hatched in three weeks; Percina, on the seventh day; Notropis, on the third day.

64. Migration of retinal pigment in Crustacea in relation to oxygen deficiency. Rudolf Bennitt and Amanda Dickson Merrick, University of Missouri. (Introduced by the Secretary of the Society.)

Overcrowding in aquaria containing crayfish results in complete distal migration of retinal pigment to the 'light-adapted' state, even though darkness always prevails. This also occurs in darkness when CO_2 is bubbled through water con-

taining only a few crayfish; thirty-four animals so treated showed complete distal migration, while twelve controls showed none; all were living after six hours when the eyes were fixed. When eighteen 'light-adapted' animals were thus treated in darkness, all remained in the 'light-adapted' condition; proximal migration did not occur. Temperature variation was kept within 3°C. Animals were then taken at intervals from CO_2 water (CO_2 bubbled at 2 to 4 liters per hour) and a sample of water was tested each time for oxygen content, CO_2 content, and pH. Complete distal migration of retinal pigment in all twelve animals occurred when oxygen content (expressed in cubic centimeters per liter) reached 0.0, CO_2 content 66.0, and pH 5.3. When CO_2 was passed through at 6 liters per hour, this occurred at oxygen content 0.0, CO_2 content 277.06, and pH 4.4. When oxygen was replaced by nitrogen (2 to 3 liters per hour), this occurred in twelve animals at oxygen content 0.0, CO_2 content 14.15, and pH 7.3. Acidification of the water with HCl had no perceptible effect until pH 3.3 to 3.9 was reached, so long as the oxygen supply remained normal.

Distal migration of retinal pigment under conditions of darkness showed no direct relationship to CO_2 content or pH, but always occurred when oxygen content reached zero in the surrounding water.

65. Some observations with the microscope centrifuge. E. Newton Harvey, Princeton University.

The microscope centrifuge is a device, recently described by Harvey and Loomis (Science, July 11, 1930), for obtaining an image of living cells with the highest dry microscope objectives while revolving 1000 to 4000 R.P.M. With this instrument the rate of stratification of granules in eggs can be observed directly, and in many cases, individual granules can be followed and are sometimes found to move in jerks as if sticking to a wall, from which they occasionally tear loose. The change of shape in the whole process of pulling Chaetopterus eggs into fragments by centrifugal force can be observed and photographed. Amoeba can put forth pseudopods (slowly) and the cilia and contractile vacuoles of Infusoria can be observed to function normally while revolving 4000 R.P.M. (2000× gravity).

66. Irradiation of the ovaries of guinea-pigs and its effect on the oestrous cycle. Ida T. Genther, Washington University School of Medicine. (Introduced by the Secretary of the Society.)

Two types of alteration in the function of the sexual organs following exposure to x-rays can be distinguished, those resulting from, 1) long exposures with filtered rays and short exposures with unfiltered rays, the latter in 40 per cent of the cases; and those from, 2) short exposures with unfiltered rays in 45 per cent of the cases.

1. Degeneration of both large and small follicles and their egg cells occurred, usually without ovulation and without the formation of a typical corpus luteum, but with hypertrophy of the theca-interna cells of the follicles to form interstitial gland. Primordial follicles continued to mature partially or completely, but without any regularity, and subsequently underwent degeneration. Oestrous cycles continued, but were irregular in length; often the oestrous period was

greatly prolonged. A large follicle (not necessarily mature) was present whenever oestrus had occurred. In the absence of the corpus-luteum action, the cycles depended on the maturation of the follicles only—therefore their irregularity and the frequent prolongation of oestrus. The genital tract and mammary gland showed typical oestrous changes; when oestrus was prolonged, their activity seemed abnormally intense.

2. Connective-tissue atresia of all the follicles occurred, except of some primordial ones; later, fibrosis of the ovary with shrinkage of the organ. Uninjured follicles developed subsequently to small follicles, but these also degenerated as soon as formed; thus no medium-sized or large follicles were ever present. Oestrous cycles were absent, and the entire genital tract was in a continuous resting condition.

67. The intracellular digestion of thymus nucleoprotein in triclad flatworms. Edward G. Kelley, New York University. (Introduced by H. W. Stunkard.)

The digestion of thymus nucleoprotein in the intestine of Planaria dorotocephala was studied by means of histological stains (iron hematoxylin and Feulgen's nucleal stain, the latter being used on hydrolyzed and unhydrolyzed sections) and the polarizing microscope.

Worms were fed on thymus gland, sectioned at various intervals after feeding, and stained. With iron hematoxylin the characteristic digestion process was noted. This involved the formation of food vacuoles in which the food material underwent a change from fragmentary particles through a finely granular stage to form transitional, then homogeneous masses which gradually disappeared. Similar changes, through the granular and transitional stages, were apparent in the food material (nucleic acid) stained with Feulgen's method. During this latter stage the vacuole material, stained by this method, decreased rapidly and disappeared at a much earlier stage than the material stained by iron hematoxylin. Unhydrolyzed sections showed nucleic-acid breakdown, but only in the digestive vacuoles.

Hydrolyzed fuchsin-stained sections (Feulgen's stain), counterstained with light green, showed numerous blue-colored vacuoles, beginning with the homogeneous stage.

Worms, in which little or no crystalline material could be seen, were fed thymus gland and examined at intervals under the polarizing microscope. A gradually increasing number of crystals was noted as digestion proceeded until a high concentration was reached at about thirty hours. This concentration did not decrease for four or more days. Preliminary solubility tests indicated unoxidized purines.

68. Some physiological effects of ultraviolet radiation on honeybees. L. M. Bertholf, Western Maryland College and Bee Culture Laboratory, U. S. Bureau of Entomology. (Introduced by S. O. Mast.)

Three series of experiments were performed to test the effect of ultraviolet on honeybees: In series I adult workers, confined in cages and fed on queen cage candy, were exposed to light from a quartz mercury-vapor lamp, used both without a filter and with each of three glass filters, one transmitting down to

313 mμ, one down to 279 mμ, and one transmitting only between 405 and 265 mμ. A wide variety of exposures was used, ranging from fifteen seconds to thirty minutes on each alternate day. Comparison of the average longevity of the bees in each cage showed, in general, that no treatment used increased the length of life significantly, but that the longer exposures decreased it, sometimes even to half that of the controls.

In series II worker and queen larvae of various ages were exposed as in series I, but here the time had to be reduced to a matter of seconds. The effect of the light was decidedly harmful and did not in these experiments result in a shortening of the developmental period nor an increase in the weight of the adults, as has been reported by other investigators.

In series III young mated queens were treated with various exposures as in series I and then placed out in colonies, each consisting of two pounds of workers. In general, no significant increase in the rate of egg laying or advantage of any other kind was observed in the treated queens, but, on the contrary, they were superseded to a considerably greater extent than the controls.

69. Reactions to light and temperature in the cercariae of the trematode Bucephulus elegans. Gerrit Bevelander, Johns Hopkins University. (Introduced by S. O. Mast.)

When the cercariae of Bucephulus elegans are subjected to a sudden increase in illumination, a complete cessation of movement occurs. This reaction does not take place immediately, but some time after stimulation. The reaction time for successive exposures at one intensity is constant. The reaction time for different intensities, within certain limits, varies inversely with the intensity. The relation between the reaction time and the intensity can be expressed by the equation $it = k$, where i = intensity, t = time, and k is a constant.

The reaction time is made up of two components, the presentation time and the latent period. Both vary inversely with the intensity.

A change in temperature results in a change in rate of rhythmical swimming movements. Between 0° and 27° a rise in temperature is followed by an increase in the rate of the rhythmical movement of the tails, and a decrease in temperature by a decrease in rate. The relation between change in temperature over this range and change in rate is fairly accurately expressed by the Arrhenius equation.

The effect of temperature on photic reaction time was ascertained for five different temperatures between 10° and 25°. Over this range the photic reaction time varies inversely with the temperature.

70. A quantitative study of reactions to electricity in Amoeba. William F. Hahnert, Johns Hopkins University. (Introduced by S. O. Mast.)

An amoeba, moving toward the anode, responds to sudden increase in current density by reversal of protoplasmic flow in the posterior end and, about a second later, by cessation of flow in the anterior end. In weak currents both are momentary, then movement is resumed in the original direction. In strong currents both persist, and movement continues in the opposite direction. The time from the closing of the circuit until response occurs, reaction time, varies

inversely with the square of the current density in both types of response. If the circuit is opened immediately after response begins, locomotion when it is resumed is in the original direction. After one reaction a certain amount of time must elapse before another can be obtained. In solutions of low conductivity, responses are more uniform and occur in weaker currents than in solutions of high conductivity.

An amoeba, moving toward the cathode, responds to sudden increase in current density by increase in the rate of locomotion at the posterior end and decreases in rate at the anterior end. The magnitude of these responses varies directly with the current density. These changes in rate persist only a few moments, then they gradually disappear. In weak currents, except for a few moments after the current is made, the rate of locomotion is not dependent upon the current density.

The electric current produces solation of the plasmagel on the cathodal side.

71. A factor modifying growth of head furnishings in Leghorn fowl. L. V. Domm, University of Chicago.

In our ovariotomized fowl minor size fluctuations occur in head furnishings from month to month, probably induced by fluctuations in hormone production. However, superimposed are marked seasonal size variations. Head furnishings almost invariably attain maximum size during winter and minimum during summer. These fluctuations are revealed by all birds in good physiological condition. Hence a gradual increase is usually observed during fall and winter, and a gradual decrease during spring and summer climaxed by molt.

Gonadal activity no doubt is an important factor governing these growth cycles, but we believe there are other vital contributory factors. One of these probably is available sunlight, for these major cycles in general coincide with periods of increasing and decreasing sunlight. An observation repeatedly made which supports this view concerns confinement. If cocks or poulardes, for instance, are confined in cages with sunlight inaccessible, they show pronounced increase in size of head furnishings. The comb may become large, thick, pale, and burdensome, so that the bird experiences difficulty in holding its head erect. When such birds are returned to pens with available sunlight, head furnishings gradually recede, ultimately becoming normal.

It is generally recognized that size and character of head furnishings in fowl are controlled by gonadal hormones. It is therefore believed that size fluctuations are directly influenced by gonadal condition. These observations reveal probable contributory factors. If we assume that the bird receives its actinic rays only, or primarily, through these appendages, increased growth tends to compensate for decreasing sunlight, and vice versa.

72. Spur dichotomy in the ovariotomized Brown Leghorn fowl. L. V. Domm, University of Chicago.

The normal female fowl rarely exhibits spurs, though these structures are present in rudimentary form. As long as the ovary retains its normal functional activity, these rudiments remain in a dormant state. However, following ovariotomy or destruction of the ovary by disease, these rudiments become activated and develop into typical spurs. In none of our ovariotomized females have spurs failed to develop.

During the course of our experiments three cases of spur dichotomy were found in our ovariotomized fowl. In two of these the left spur only was dichotomous, the right being normal, while in the third case both right and left spurs were dichotomous. In the two cases showing only left-spur dichotomy, these were nearly of equal size and as large as the normal single right spur. In one of the pair the proximal was largest, in the other the distal. In the case exhibiting both right and left dichotomous spurs there was considerable size discrepancy, the proximal of each pair being considerably smaller. These were curved, pointed, and triangular instead of circular structures. In no case were the dichotomous spurs grown together.

All three females were secured from the same source, two of them belonging to the same hatch. They were sinistrally ovariotomized when forty-three, fifty-six, and fifty-seven days old, respectively. A comparable condition has never been found in our normal or castrated males. It is probable that a developmental arrest at an early stage during the formation of the spur rudiments may be responsible for the condition.

73. A demonstration of equivalent potentialities of right and left testis-like gonads in ovariotomized fowl. L. V. Domm, University of Chicago.

An invariable sequel of sinistral ovariotomy has been hypertrophy of the rudimentary right gonad usually into a testis-like gonad. A similar gonad may develop simultaneously on the left, though is encountered much more infrequently. Experiments were devised to demonstrate the reciprocal endocrine potencies of these gonads. Sinistrally ovariotomized individuals were subsequently dextrally ovariotomized and the left testis-like gonads allowed to develop.

Birds with only left testis-like gonads develop masculine head furnishings. Variations in size occurred at different periods, interpreted as due to their dependence upon the size and condition of the gonad. Following operation, plumage became male, but subsequently reverted to female. Some revealed secondary reversions to male, followed by reversions to female interpreted as due to fluctuations in production of female hormone which consequently ranged above and below the threshold of effectiveness. Two cases had not revealed initial reversion from male to female at termination of experiment. All individuals revealed some masculine behavior, particularly pronounced in one case, though none were known to tread. Spurs developed. The wolffian ducts revealed readily perceptible growth, in some becoming definitely convoluted. Oviducts varied in size. The largest were found in birds having reverted female plumage, the smallest in those having revealed no indications of such a change.

All modifications observed in birds of this series, revealing only left testis-like gonads, coincide very closely with those previously observed in sinistrally ovariotomized birds revealing only right testis-like gonads and demonstrate quite conclusively equivalent endocrine potentialities of right and left testis-like gonads in ovariotomized fowl.

74. *Respiratory metabolism during pupal development of Galleria mellonella.* Ivon R. Taylor and H. B. Steinbach, Brown University. (Introduced by H. E. Walter.)

Throughout the entire period of metamorphosis, practically continuous measurements of the rates of oxygen consumption and carbon-dioxide production of Galleria mellonella (bee moth) have been made on the pupae individually by means of sensitive differential manometers at a constant temperature of 30°C. The rates were found to be high at first, to decrease steadily to a minimum, then to increase to a maximum, and finally to decrease just prior to emergence. During the greater part of metamorphosis the rates of the males, on the average, exceed those of the females, but throughout the last quarter of pupal development the rates of the females, on the average, are higher than those of the males, giving a statistically significant sex difference in the minimum and maximum rates of oxygen consumption. These rates have been expressed on the basis of 1 gram weight of the entire pupa. It has not been possible to do this on the basis of the actual amount of organized tissue present because histolysis and histogenesis occur during metamorphosis. This sex difference may be due to a difference in the relative amounts of organized tissue present, and not to any fundamental difference in the metabolism of males and females. The respiratory quotients have been determined at frequent intervals throughout metamorphosis and have been found to have the average value of 0.69, with very little variation during the entire pupal period.

75. *Effect of injection of anterior-pituitary extract on the thyroids of mice with hereditary dwarfism.* George D. Snell, Dartmouth College and Brown University. (Introduced by A. M. Banta.)

'Dwarf' is a recessive mendelian character in mice causing practical cessation of growth after the fourteenth day and complete sterility in both males and females (Snell, '29). The thyroid of the dwarfs is reduced in size and interspersed with connective tissue, and the follicular epithelium is conspicuously flattened.

A study has been made of the effect of daily subcutaneous injections of an anterior-pituitary extract prepared by Armour & Co. and stated by Uhlenhuth ('28–'29) to be a specific thyroid stimulator. In three treated dwarfs the thyroids show marked signs of restored activity. The follicular epithelium is cuboidal or columnar and the connective tissue is somewhat reduced. Treated individuals show an average gain in weight of about 50 per cent, as compared with the controls, and a marked increase in general activity. The opening of the vagina was not significantly hastened in three treated females. One of two treated males was fertile, giving a litter of three dwarfs and one normal when mated to a heterozygous female.

Thyroid feeding of four individuals caused a gain in weight, increased activity, and a marked lengthening of the hair as compared with the controls. One thyroid-fed male was fertile.

76. An experimental study of the factors concerned in mammary growth and milk secretion. W. O. Nelson and J. J. Pfiffner, Washington Square College, New York University, and the Biological Laboratory, Cold Spring Harbor. (Introduced by H. O. Haterius.)

Daily injection of a lipid extract of sow's corpora lutea into normal and castrated immature male and spayed immature female guinea-pigs produced a very marked hypertrophy of the mammary glands and the nipples. However, no milk secretion occurred, even in animals injected over a period of thirty days, unless the lutein treatment was followed by injection of either fresh anterior-pituitary substance or its extract. In such cases lactation began within three days.

Injection of the anterior-pituitary principle into immature animals not previously treated with the lutein extract failed to initiate milk secretion. A copious flow of milk was produced in mature females (normal or spayed) within three days by treatment with the pituitary extract only. This latter is in agreement with Corner's observation on the rabbit.

Control tests have shown the oestrin content of the lutein extract to be negligible. Its presence in minute amounts is probable, but the amount is insufficient to account for the growth obtained. Previous work has demonstrated the potency of this extract with regard to its content of the corpus-luteum hormone.

These results indicate that, in the guinea-pig, the mammary glands must first be developed to a proper state before the pituitary principle can initiate secretion. On the other hand, ovarian factors alone are not sufficient.

77. Anterior-pituitary therapy in spontaneous ovarian dysfunction. W. O. Nelson, Washington Square College, New York University. (Introduced by H. O. Haterius.)

While following the vaginal smears of several hundred rats, eight animals were found which for a period of at least five weeks showed no oestrous changes whatever. These animals were apparently in the best of health and quite normal in every respect except for their reproductive cycle. Age, inanition, and vitamin-B deficiency could be ruled out as causative factors. The possibility suggested itself that some factor in the normal pituitary-gonadal relationship was inactive.

One ovary and a portion of the uterus were removed in each instance and the animal allowed two weeks for recovery. During this period no oestrous changes occurred. Intramuscular injection of one fresh anterior pituitary daily induced a typical oestrus within four days. Autopsy (in four instances) on either the day of oestrus or the following day demonstrated the usual oestrous picture in the ovaries and uteri. Histological study of the ovaries showed either many ripe follicles or numerous fresh corpora lutea. Two of the females were bred and bore normal litters. Control injection of muscle tissue in two cases elicited no response. Injection of the pituitaries of two of the animals, after oestrus had been induced and a two-week period of anoestrus had subsequently occurred, caused oestrus and ovulation in a third animal. This indicated that the maturity hormone was present in the pituitary, but apparently was not being liberated.

These results are suggestive of the possible clinical application of anterior-pituitary therapy in some types of sterility and gonadal dysfunction.

78. The effect of ovarian implants on castration-cell types in the male rat pituitary. H. O. Haterius and W. O. Nelson, Washington Square College, New York University.

In an attempt to determine whether or not gonads of the opposite sex are capable of preventing the appearance of the so-called 'castration-cell' types in the anterior pituitary, a series of transplantations has been carried out involving implantation of rat ovaries into males accompanied or followed within a few days by castration. Autopsy, at least a month subsequent, revealed, out of fourteen cases, eight successful takes and six in which no ovarian substance could be demonstrated, as evidenced by microscopic examination. Histological study of the pituitaries revealed in each instance of a successful take a total absence of castration-cell types, whereas in the cases of non-takes the typical castration-pituitary picture was found. A second series was run, to determine whether ovarian implants are capable of correcting the histological picture of the pituitary once castration cells have developed. Male rats (litter mates), castrated four months previously, were implanted and allowed to live a month before autopsy. Controls were castrated at the same time as the experimental animals and sacrificed at the time of implantation. Results to date indicate that ovarian substance is capable not only of preventing, but also of correcting, the castration-cell picture of the male anterior pituitary.

These results are not in agreement with those of Lehmann, who reported a definite sex specificity in so far as gonadal control over the appearance of castration-cell types is concerned.

79. Vaginal cornification and ovarian blood supply. H. O. Haterius, Washington Square College, New York University.

A series of normal female rats subjected to subtotal oophorectomy (all but a fragment of one ovary being removed) displayed excessive cornification of the vagina for periods of four to eight months—until time of sacrifice. Such cornification manifested itself in extended periods of characteristic oestrous smears, interspersed with an occasional typically pro-oestrous one. Binocular examination of the ovarian region at autopsy revealed a marked increase in blood supply, as evidenced by an extensive branching and an increase in size of the ovarian blood vessels. The hypertrophied ovarian fragment in each instance displayed several large cystic follicles. As a check on the possibility that the increased blood supply might be the direct causative factor for excessive cornification, the ovarian vessels of a series of normal animals were painted with 5 per cent phenol. Such treatment resulted in a pronounced engorgement and dilatation—a condition which remained persistent as revealed by subsequent examination. A continuous oestrous smear was displayed for several months following treatment and, as in the first series, the ovaries at autopsy revealed large cystic follicles. Controls, treated with normal saline, were negative in this respect. In neither series was the mating reaction present, indicating that the condition is not a true oestrous reaction.

80. The influence of ferrous iodide and oleic acid on rats depleted of vitamin A. F. E. Chidester, A. G. Eaton, and N. K. Speicher, West Virginia University.

Withdrawn.

81. The experimental production of pregnancy cells in the pituitary gland of mice. I. The occurrence of pregnancy cells in female mice following continuous anterior-lobe administration. H. O. Haterius and H. A. Charipper, Washington Square College, New York University.

During pregnancy the anterior pituitary gland is characterized by definite cell types, 'Schwangerschaftszellen,' and the ovary is predominantly luteal. It seemed possible that pregnancy cells could be demonstrated following the experimental development of a dominant luteal phase in the ovary of non-pregnant animals by means of continuous implants of anterior lobe. Normal, non-pregnant mice were given a total of nine daily implants of anterior pituitary (rat). The pituitaries and ovaries were then sectioned and compared with the corresponding glands removed from a pregnant animal. The ovaries of the experimental animals were decidedly luteal, giving a histological picture similar to that of ovaries of pregnancy. The anterior pituitaries from the experimental mice contained typical cells of pregnancy: large ovoid cells containing a vesicular nucleus eccentrically located in a mass of homogeneous cytoplasm which stained deeply with eosin. These cell types appear to be identical with those found in the anterior pituitary of pregnancy.

82. The experimental production of pregnancy cells in the pituitary gland of mice. II. The occurrence of pregnancy cells in male mice following continuous anterior-lobe administration. H. O. Haterius and H. A. Charipper, Washington Square College, New York University.

It seemed of considerable theoretical interest to determine whether or not the male pituitary possesses the potentiality of developing a pregnancy-cell type when furnished a suitable stimulus. The procedure was initiated by castration with simultaneous implantation of ovaries in the scrota, which was followed by nine daily administrations of fresh anterior lobe (rat), on the principle employed previously in a series of females. Histological study of the recovered pituitaries demonstrated the occurrence of typical pregnancy cells, identical with those previously found in females. This condition was found constant in each case in which the graft had taken. The grafts displayed a marked development of luteal tissue. Further, it is probable that castration is not essential, since in one instance one testis was left in situ; nevertheless, the graft was successful, and the pituitary of the host displayed the typical picture of the pituitary of pregnancy.

83. The sensitivity of the legs of certain Calliphoridae to saccharose. Selma Crow, University of Minnesota. (Introduced by D. E. Minnich.)

A series of experiments, based on the technique developed by Minnich, was performed to determine the thresholds of the right fore legs of Lucilia sericata, Calliphora erythrocephala, Calliphora vomitoria, and Cynomyia cadaverina to saccharose during a period of water diet lasting until death. All four species of flies readily distinguished water from saccharose solutions of appropriate concentrations by means of their fore legs. As the period of water diet progressed, the thresholds of response to saccharose fell, the final low levels being reached shortly before death. The manner in which they fell was very similar to that observed by Minnich for the nymphalid butterfly, Pyrameis atalanta, and the blowfly, Calliphora vomitoria. The lowest thresholds of response recorded for the

various species were as follows: Lucilia, 1/3200 M — 1/800 M; C. erythrocephala, 1/3200 M — 1/1600 M; C. vomitoria, 1/12,800 M — 1/3200 M; and Cynomyia, 1/25,600 M —1/6400 M. To the lower concentration in each case the animal failed to respond, while to the upper it was consistently responsive.

84. The sensitivity of the oral lobes of the proboscis of the blowfly, Calliphora vomitoria Linn., to various sugars. Dwight Elmer Minnich, University of Minnesota.

Using proboscis extension as an index of reception, the sensitivity of the oral lobes of the blowfly, Calliphora vomitoria, has been studied with respect to certain sugars. If maintained in a state of satiety with respect to water, contact of the oral lobes with water results in few or no responses, as a rule. Contact with 64/100 M saccharose, however, yields 100 per cent response. During a period of water diet lasting until death, the threshold of response to saccharose falls little, if any, and the minimum concentration which will effect a proboscis extension in the last extremity of inanition appears to be between 1/400 M and 1/100 M. The order of effectiveness for the sugars tested was found to be: saccharose (=)[1] maltose>glucose>lactose, the last being relatively ineffective. In concentrations of 64/100 M or 128/100 M, however, lactose will effect vigorous responses in animals which have been subjected to seventy-two hours of water diet. Compared with the chemoreceptors of the legs, the order of effectiveness for the above sugars is the same. There are two important differences, however, between the chemoreceptors of the mouthparts and those of the legs. First, the oral lobes appear to be much less sensitive to saccharose, being only about one-sixteenth as sensitive as the legs in the last extremity of inanition. Secondly, the oral lobes are sensitive to lactose in high concentrations, while the legs are insensitive.

[1] Approximately equal.

85. Hydrogen-ion concentration of the amniotic and allantoic fluids of the pig. Charles E. Abromavich, Jr., Johns Hopkins University. (Introduced by the Secretary of the Society.)

A study of the allantoic and amniotic fluids of the pig embryo has shown that there is a difference in pH between the two fluids and, furthermore, that a difference is maintained between these fluids throughout gestation. Considerable individual variation was found in the pH of the amniotic fluid, while the allantoic fluid showed very little diversity. The amniotic fluid manifested a general trend toward increased acidity as gestation advanced, and during the latter stages, in several instances, the amniotic fluid had the same acidity as the allantoic fluid, i.e., pH 6.4. The range of acidity for the amniotic fluid was from pH 7.5 to pH 6.4, while the range for the allantoic fluid was from pH 6.5 to pH 6.2.

Pregnant uteri were obtained from a local slaughter house and fifty litters of various stages of gestation were examined. The uteri contained embryos varying from 18 mm. to 250 mm., crown-rump measurement. The hydrogen-ion concentration was determined colorimetrically and such precautions as, e.g., non-exposure to atmosphere, etc., were observed.

In view of the fact that the data demonstrate a difference in the pH between the amniotic and allantoic fluids, it might be suggested that we are dealing with a Donnan phenomenon and that the amniotic membrane serves as a regulatory membrane to maintain this difference.

EMBRYOLOGY

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86. Viability of Physa egg fragments. E. D. Crabb, University of Colorado. (Lantern; 10 min.)

The operated egg and a control in the same cleavage stage were studied and allowed to develop in a culture slide having a 1-ml. capacity. Fresh water was supplied by means of a wick siphon. Using Fry's ('24) glass-needle technique, the vitelli of thirty-one Physa gyrina eggs were successfully injured in the two-cell and in the four-cell stages. After destruction of a primary blastomere, the uninjured blastomere usually continued to develop five to forty hours; in one case, 167 hours. The oldest of these half-eggs formed characteristic double segmentation cavities before dying. Very slight injury to one of the primary blastomeres resulted in delayed, abnormal cleavage and ultimate death of the embryo. Death resulted from injury to one or two cells in the four-cell stage. It was impossible to injure the vitelli with any degree of accuracy, due to their being suspended in albuminous fluid. Thus at least 90 per cent of the eggs were hopelessly mutilated.

87. A possible relationship between spermatozoon age and foetal abnormalities in the guinea-pig. William C. Young, Brown University.

Withdrawn.

88. The postembryonic development of mendelian characters in the goldfish Carassius auratus. H. B. Goodrich and I. B. Hansen, Wesleyan University. (Lantern; 15 min.)

The development of the mendelian color characters of goldfish that have been described by Chen ('28) is traced with special reference to the melanophores and reflecting tissue. Until the 12-mm. stage the history of melanophores is identical in the common goldfish, the transparent shubunkin and the heterozygous type or true shubunkin. After this stage the number of melanophores increases in the common goldfish, are destroyed in the transparent shubunkin, and show an intermediate condition of partial growth and destruction in the hybrid. The results are numerical, based on counts of melanophores. In the common goldfish as shown by previous work the melanophores also degenerate much later in ontogeny (one and one-half to seventeen months).

In the common goldfish reflecting tissue begins development in certain definite loci from where it spreads until the whole body, excepting fins and pupil of eye, is covered. In the transparent fish very little is developed. In the heterozygous form development starts on the same loci as in the common fish, but growth is early and irregularly inhibited. The total of reflecting tissue of the hybrid is intermediate in amount between that of the two homozygous types.

89. Origin of the aortic arches in Amphibia. Waldo Shumway, Selma M. Olson, and Otto E. Kugler, University of Illinois. (Lantern; 10 min.)

In the course of investigations on amphibian metamorphosis we have encountered some evidence concerning the theory suggested by Boaz ('82) and recently by Figge ('30) that the absence of metamorphosis in Necturus is associated with an absence of the sixth aortic arch. In Amblystoma microstomum five arches appear, of which the first ('hymandibularis,' Maurer) represents the classical mandibular arch (I); II is absent. III, IV, and V follow the typical course of development described by Maurer in Triton. VI gives rise to the pulmonary arch. It is represented dorsally by a twig from the third efferent trunk at 8.5-mm. body length and ventrally by a twig from the truncus arteriosus in the fourth branchial arch, which unites with the dorsal portion at 12-mm. body length. In Necturus maculosus traces of all six arches appear. In addition to the 'hymandibular' arch (I) there is a vestigial dorsal vessel (II) in the hyoid arch of embryos 9 to 10 mm. in length. III, IV, and V arise as in Amblystoma. The last arch (VI) is represented by a vestigial ventral twig found in embryos between 20 and 40 mm. in body length, and a dorsal twig arising as in Amblystoma and from which the pulmonary artery originates.

90. Differential counts of the leucocytes of fetal albino rats. J. E. Kindred, University of Virginia. (Lantern; 15 min.)

This is a preliminary account of the percentage of types of leucocytes of fetal rats observed in blood smears stained with Wright's stain. Blood smears from rats averaging 15.4 mm. to 43 mm. in length (C–R) have been made. The period of gestation included between these stages is the last third. The differential classification includes: hemocytoblasts; myelocytes; neutrophilic, eosinophilic, and basophilic granulocytes; small and large lymphocytes, and monocytes. The hemocytoblasts comprise the largest group in the earliest stage, averaging 50 per cent of the cells identified; granulocytes are relatively scarce, about 15 per cent; lymphocytes average about 35 per cent. As the fetuses grow older the relative percentages of the several types of leucocytes change. The hemocytoblasts decrease in number, until at birth they are practically lacking in the circulating blood. Myelocytes are most numerous at the 25-mm. stage. Small lymphocytes are numerous at the 25-mm. stage, and remain at about the same level throughout the duration of gestation. Neutrophilic granulocytes show a great increase just before birth. Monocytes are practically negligible throughout.

91. The sugar content of the blood of the fetal albino rat. E. L. Corey, University of Virginia. (Lantern; 10 min.)

The blood-sugar content of 108 fetuses of from 13- to 40-mm. crown-rump length was determined. Of these, forty-seven were males, fifty-seven females, and four undetermined as to sex. The average fetal blood sugar was 69 mg. per cent, while the maternal blood sugar for sixteen litters was 112 mg. per cent. Blood samples were obtained from the jugular vein of the fetus in all cases, the mother being stunned by a blow on the head. By this means the mother was maintained alive and all fetuses were viable at the time the blood samples were drawn. At the 13-mm. stage (the youngest stage at which adequate blood samples

could be obtained) the average blood-sugar content of the fetuses was much lower than that of the mother, e.g., 60 mg. as compared to 85 mg. per cent. Noteworthy differences in fetal blood sugar are observed at subsequent age periods. A decided rise occurs from the 32-mm. stage onward. At term the fetal level approximates that of the mother. A correlation between the levels of the fetal and maternal blood sugar is evident throughout the series. The average blood-sugar content of pregnant albino rats is higher than that of non-pregnant animals. No differences were apparent, however, between non-pregnant female and male control animals.

92. Micromoving pictures showing the embryonic circulation. Bradley M. Patten, Western Reserve University, and (by invitation) Theodore C. Kramer, Baldwin Bird Research Laboratory. (Motion-picture projection; 15 min.)

Moving pictures of living chick embryos during the second day of incubation. Cuts from films showing the spasmodic initial contractions of the heart, the beginning of the movement of blood corpuscles in the vessels, and various views of the circulatory mechanism after it has settled into regular rhythmic activity.

93. Bisexuality and sex differentiation in embryos of the musk turtle, Sternotherus odoratus (Latreille). Paul L. Risley, University of Michigan. (Introduced by Peter Okkelberg.) (Lantern; 10 min.)

The examination of a series of S. odoratus embryos during an investigation on the origin of the germ cells revealed a condition hitherto undescribed in studies on gonadogenesis in Amniotes. During the formation of the indifferent gonads, the primordial germ cells are distributed approximately equally to the medulla and the sex cords, and to the germinal epithelium (embryos 5.0- to 6.0-mm. carapace length). Shortly thereafter, the germinal epithelium increases in thickness mainly through rapid multiplication of the germ cells, and forms a definite cortical layer (ovarian) of variable proportions.

Immediately after the multiplication period, many germ cells of the cortex enter into synizesis, most of them persisting in the ovary and entering the growth period shortly before hatching. In the ovary the medullary portion degenerates. In the testis the sex cords develop into the seminiferous tubules and the cortex degenerates; however, all male gonads examined from this period up to sexual maturity present some indication of their early bisexual character. The presence of the accessory sex organs of both sexes confirms the bisexual state of these embryos.

The bisexual character of the gonads is regarded as a morphological expression of the presence of both male and female sex-differentiating substances within the embryo; a morphological foundation for the development of every embryo in either the male or female direction is present during the bisexual period.

A preponderance of males indicates that, in some of these laboratory embryos, a sex reversal has occurred, the causal factors of which are unknown.

94. Studies of living nerves. I. The movements of sheath cells and nerve sprouts, correlated with the process of myelin formation in amphibian larvae. Carl Caskey Speidel, University of Virginia Medical School. (Lantern; 15 min.)

The process of myelination of nerve fibers has been watched in living frog tadpoles. The movements of individual nerve sprouts and the migrations of individual primitive sheath cells of Schwann have been followed for many days and correlated with the addition of new myelin sheath segments. Some tentative conclusions are:

1. A 'myelin-emergent' nerve sprout differs from a 'non-myelin-emergent' fiber. The former in combination with a primitive sheath cell leads to the formation of new myelin segment, the latter ordinarily does not.

2. Early unmyelinated nerves serve to direct advancing myelin-emergent nerve sprouts and to furnish them with primitive sheath cells. The acquisition of primitive sheath cells by myelin-emergent sprouts is greatly expedited by the movements of the sprouts in expansion, retraction, branching, and the formation of temporary anastomoses with adjacent fibers.

3. The transfer of a primitive sheath cell from non-myelin-emergent fiber to a myelin-emergent fiber may be effected in a variety of ways. The reverse transfer, however, i.e., from a myelin-emergent fiber to a non-myelin-emergent fiber, has never been seen. Transfer from one myelin-emergent sprout to another may also take place.

4. The occasional occurrence of non-myelinated gaps between adjacent myelin segments is rectified by the process of intercalation of a horizontal myelin segment. Addition of a perpendicular myelin segment may occur at a node of Ranvier or at the end of the terminal myelin segment.

Direct visual evidence is also presented in support of many, but not all, of the current tenets of neurogenesis.

95. Further experimental studies of the opercular structures in Hydroides dianthus. Charles Zeleny, University of Illinois. (Lantern; 10 min.)

In Woods Hole species, Hydroides dianthus, as in the Naples species, H. pectinata, upon which a previous report was made, the character of the opercular differentiation is a function of the age at which the stimulus is applied, and not of the materials upon which the stimulus acts. Gill, stump of gill, and rudimentary operculum produce identical functional opercula when stimulated at the same age. These facts taken in connection with those of the normal ontogeny of Hydroides and the adult opercular characteristics of other genera of the Serpulidae suggest that evolution within the group may have proceeded in part by changes in genes affecting the relative rates of different developmental processes.

96. Tail induction through the hind parts of the neural plate in Triton. H. Bytinski-Salz, Yale University. (Introduced by R. G. Harrison.) (Lantern; 15 min.)

Either one of the posterior two-fifths of the neural plate grafted into the blastocoel of a Triton gastrula has the capacity for self-differentiation—the more cranial fifth to neural tube, the caudal fifth, including the blastopore lips, after

invagination, into myotomes and lateral mesoderm. These materials are distinguished from the more anterior parts, both of the neural plate and the mesoderm, by their possibilities to stretch or bud and through a general power to regulate the form of lost parts.

These portions may induce a neural plate, ear vesicles, sense placodes, and also a tail; this tail induction is independent of the induction of a neural plate and lacks the anterior part of the latter. In the induced tail the graft may form neural tube, myotomes, and notochord, while the host tissue may be induced to form neural tube, myotomes of tail bud, tail fin, typical mesenchyme structure in the fin, and two rows of chromatophores. Notochord has never been induced.

The inductive influence of the graft on the ectoderm and the mesoderm of the host is unquestionable; there is, furthermore, an influence, probably an inductive one, on the entoderm in the formation of a process of the ventral wall of the gut which grows into the induced tail.

These experiments demonstrate that the formation of the tail in normal development shows a double, perhaps multiple, form of assurance, because both the myotomes and the neural tube of the tail (which is itself induced by the notochord of the tail) can induce a tail.

97. The reproductive system in half-grown western axolotls (Amblystoma tigrinum?) following hypophysectomy. R. K. Burns, Jr., and Adrian Buyse, University of Rochester, School of Medicine and Dentistry.

A considerable number of half-grown Colorado axolotls, hypophysectomized in the course of other experiments, were reared in order to study the effects of hypophysectomy on the reproductive system, metamorphosis, and other functions. Nineteen of these animals were preserved at intervals throughout a period of six months; others still survive and will be kept indefinitely. The principal effects noted may be briefly listed:

1. Metamorphosis is at once inhibited, although the animals continue to grow and in many cases attain maximum size.

2. The typical silver-white color characteristic of hypophysectomized amphibians is assumed within a few hours.

3. A modified fat metabolism leads to a general adiposity, with great enlargement of the fat-bodies. This does not appear to be correlated with sex.

4. In males the gonads appear to cease developing almost at once. In six months the testes are scarcely larger than at hypophysectomy, although the animal has grown several centimeters in this time. They appear even to degenerate, becoming slender, thin, and leaf-like. In the females the ovaries are much less inhibited and approach the normal in size. The ova grow to large size and the ovary becomes pigmented. There is individual variation as to the amount of degeneration or development that has occurred, and the development of the ducts appears to be correlated with this.

98. The occurrence of abdominal organs in the thoracic cavity in the domestic cat. H. H. Collins and Jean MacCreight, University of Pittsburgh. (Lantern; 7 min.)

The entire small intestine occurs within the left pleural cavity, along with the greater portion of the great omentum which is ventral to the intestine with its

free border extending craniad to the level of the first rib. A small portion of the omentum passes into the right pleural cavity through a perforation in the mediastinal septum caudad of the heart. Both lobes of the pancreas are entirely within the left pleural cavity. The left lung is abnormal in form, displaced dorsally, and very much reduced in size. The right lung is more nearly normal, but is displaced dorsally. The heart lies to the right of the midventral line.

In the abdominal cavity the stomach is displaced to the left and twisted so that the greater curvature lies along the midventral line. The pylorus is directed craniad and to the left. The duodenum passes craniad through a large foramen in the diaphragm. The descending colon passes through the same aperture to the abdominal cavity, where it continues as a straight tube to the anus. The liver is markedly displaced to the right, the stomach and spleen to the left, in the cranial portion of the abdominal cavity.

The specimen is fully grown and in perfect condition. Apparently the animal was not at all handicapped by the abnormal arrangement of its visceral organs.

99. A twin-headed human fetus. R. A. Muttkowski and Leo E. Buss, University of Detroit. (Presented by Leo E. Buss; introduced by R. A. Muttkowski.)

A full-term twin-headed human fetus was obtained in one of the Detroit hospitals. Preliminary examination showed two separate hearts, two stomachs, three arms, two heads, two legs. X-ray photographs show two complete spines, but only one pelvis. The middle arm shows two humeri, and a single bone for the radius-ulna.

By demonstration

100. Internal relations found in twins and double monsters produced by ultraviolet radiation. Marie A. Hinrichs, University of Chicago.

During four summers' study at Woods Hole, 179 cases of twins and double monsters were experimentally produced by means of early exposure of fertilized eggs of Fundulus heteroclitus to ultraviolet radiation. Of these, eighty-nine cases were sectioned and studied, and the following conditions of internal organs noted: the incidence of situs inversus in double monsters and in twins; occasional independent lens formation; organ duplicities; differential inhibition in autosite-parasite monsters, and asymmetrical relations in the two components of double monsters.

101. A bovine freemartin. R. K. Burns, Jr., and Adrian Buyse, University of Rochester, School of Medicine and Dentistry.

The reproductive system of a bovine freemartin, aged two and one-half years, proved to be modified to an unusual degree. This animal was one of a set of triplets, the other two members being normal males. The external genitalia, so far as visible, were of the usual freemartin (female) type; the physique was masculine. The vulva was small, with a very narrow vestibular cleft leading into a narrow urinogenital sinus some 5 inches in depth. No traces of vagina or uterus could be found. The urinogenital sinus received the opening of the urethra in the midline, while laterally, large lobulated seminal vesicles opened. The vasa deferentia were prominent and convoluted distally, but it is uncertain whether a

lumen is present in the proximal part. The gonads were very small and were found in the inguinal canals, each possessing a typical epididymis. A histological study of the gonad has not yet been made. Most striking was the presence of a penis several inches in length, which was found in a greatly contorted condition, lying beneath the ventral wall of the vestibule and urinogenital sinus. Distally, it penetrated the wall of the vestibule and terminated in a large glans penis, which protruded through the lower angle of the vestibular cleft. So far as is known to the writers, only a case reported by Numan ('43) exhibits a like modification of the external genitalia (Lillie, '17).

By title

102. The nervous structure of the annelid ganglion. W. M. Smallwood, Syracuse University.

The two-cell reflex has played an important part in the explanation both of the working and evolutionary history of the nervous system. The nervous system of the earthworm has been cited for nearly forty years in support of the two-cell reflex. A more critical study of the distribution of the nerve cells within the ganglion and their synaptic relations clearly indicates that the two-cell reflex has no place in connection with this group of cells. The peripheral distribution of these same cells gives further emphasis to this same conclusion. The distribution within the ganglion of the sensory fibers is very extensive and distributes incoming impulses to a great many cells. The third important fact in rejecting the two-cell reflex is the part played by the giant fibers and their connections with the motor cells.

103. The development of the carp. Study no. 1. The larval life of the carp, with special reference to the development of the intestinal canal. W. M. Smallwood and Mary Lovett Smallwood, Syracuse University.

This is the first of a series of studies that is being carried out on the development of the carp. The authors have collected the eggs of carp, hatched them, and observed the development through successive stages, covering many months. The larval life of the carp falls into, 1) the inactive stage, lasting from four to five days, and, 2) the active stage, when the yolk sac has become largely absorbed and food is captured. The intestinal epithelium is formed about two days before hatching. In the period just when the larva is escaping from the egg envelope and the following twenty-four to thirty-six hours, the intestinal epithelium undergoes transition from the simple columnar stage to the beginning of the adult plan.

104. The development of the carp. Study no. 2. The development of the ear and lateral-line organs. W. M. Smallwood and Phyllis Virginia Plyler, Syracuse University.

The lateral-line cells are clearly differentiated in the early larval period and the lateral-line nerve can be demonstrated terminating around the base of the lateral-line cells in the late larval period. The ear first appears as a thickening of the deeper layer of the ectoderm which appears soon after the closing of the neural tube and before the hatching of the embryo. The various stages in the formation of the ear have been determined and the lateral line in the head as well as the trunk region.

105. The aortic arches and their derivatives in the embryo porcupine (Erethizon dorsatus). Parke H. Struthers, Syracuse University. (Introduced by W. M. Smallwood.)

This ontogenetic study shows the following facts: 1) the presence of a vidian artery homologous with that of reptiles, which serves visceral elements of the jaw; 2) there exists a transitory occipital artery arising from the stapedial which contributes to the vascular supply of the occipital region; 3) the presence of a transitory fifth arch intimately associated with the sixth arch; 4) there is evidence of at least two presegmental branches of the aorta; 5) in the development of the adult pulmonary stem the right artery forms very little of the common vessel; 6) a single cephalobrachial trunk forms the culmination of arch development; 7) arterial development of the head and neck falls into three phases: *a*) a temporary arterial pattern designed to carry nutriment to primitive head structures, *b*) a plan of arterial distribution adapted to supply the rapidly forming cartilage and muscle of the jaws, and, *c*) a readjustment period when the arterial plan is readjusted, due to the increased heteronomy of the head. (Complete article to appear in the Journal of Morphology and Physiology.)

106. Effects on the nervous system following the embryonic transplantation of the spinal cord in the anuran, Discoglossus pictus Otth. Raoul M. May, Collège de France and Institut Pasteur.

A piece of embryonic spinal cord, with the surrounding tissues, was transplanted homoioplastically in embryos of Discoglossus pictus Otth., at the tail-bud stage, in the hind-limb region.

When the implanted spinal cord developed independently of the hind limb on the same side, the autochthonous spinal cord and its ganglia had a symmetrical structure. But in certain cases the implantation inhibited completely the development of the homolateral hind leg, while in two cases the grafted spinal cord gave rise to a large, well-formed nerve, which usurped the functions of the normal lumbosacral plexus and innervated the homolateral hind leg. In all these cases of absence of innervation to a hind limb from the autochthonous spinal cord there is a reverberation on the latter and its ganglia through a sensory and motor hypoplasia. The ganglia are very much reduced, and the lateral half of the spinal cord undergoes a marked reduction of its white and gray matter—on the side and along the segments from which the lumbosacral plexus is absent. The spinal-cord reduction is seen through the absence of the motor horn and by a marked asymmetry. There is also a repercussion of the reduction in the neighboring segments.

These results show that the hind-limb innervation plays a part in the development of both the sensory and motor neurones of the neuraxis.

The complete paper, with results expressed quantitatively, will appear in the Bulletin Biologique de la France et de la Belgique, T. 64, fasc. 3, 1930.

107. Transplantation of cerebral tissue of the newborn rat into the eye of the adult albino rat. Raoul M. May, Collège de France and Institut Pasteur.

Cerebral tissue of newborn albino rats was introduced into the anterior chamber of the eye of adult albino rats. The fragments of cerebral tissue resisted to asphyxia, became grafted onto the iris, were vascularized, and remained alive as long as the host rats were kept—nearly six months for some of them.

In some cases the nervous grafts became intimately applied to the internal surface of the cornea, but had no vascular or nervous connections with the latter. In all cases a meninge was formed around the graft. The nerve cells of the transplants had a structure which was essentially that of normal cerebral cells; there were also present neuroglia cells.

The typical arrangement of cerebral cells did not seem to exist in the grafts, throughout whose mass they were dispersed. The nerve fibers of the iris and those of the transplant remained in their respective tissues; there was thus no nervous interpenetration between the graft and its host.

These results show that the grafted nervous fragment becomes perfectly adapted to its new environment and that it retains its specificity.

The complete paper will appear in the Archives d'Anatomie Microscopique, T. 26, fasc. 3, 1930.

108. The origin of the rete testis in the domestic fowl. J. C. Gray, Western Reserve University.

The origin of the rete cords in birds has been ascribed to three different tissues by previous investigators. A continuous series of embryos from two days through hatching was studied as well as posthatching stages of one, two, and four weeks of age. After sex differentiation only males were studied. The rete testis arises from cords of cells lying between the gonad and wolffian body. These rete cords are derived from the distal ends of some of the sexual cords of the first proliferation. They may or may not separate from the sexual cords. Usually the rete cords contain germ cells. The original rete cords give rise to secondary rete cords which later receive the distal ends of other sexual cords. The differentiated rete lies between the gonad and the wolffian body.

Shortly after hatching, the rete cords begin to differentiate into channels lined with low cubical epithelium. They are nearly complete by one month after hatching. At this time the tubules of the wolffian body differentiate into efferentia and epididymal tubules. A portion of the anterior part of the wolffian body is enclosed within the suprarenal capsule and remains there when the former becomes the epididymis.

109. On the process of incorporation and vascularization of an implant by the chorio-allantoic membrane. Lillian O. Henderson, University of Chicago. (Introduced by B. H. Willier.)

The area pellucida of definitive primitive-streak stages of chick embryos was transferred to the chorio-allantoic membrane of host embryos incubated eight, nine, and eleven days. The grafts were removed at intervals from five to ninety-six hours. Through a study of these grafts the process of incorporation and of vascularization as well as the relation of the invading mesenchyme and blood vessels to the developing organs of the implant were determined.

Five hours after transplantation the implant has become fused to the membrane and a definite thickening of the underlying ectoderm and mesenchyme is noted. As the graft sinks into the mesenchyme, cells proliferated from the ectoderm at the margin of the grafted blastoderm extend over its upper surface and finally, at the end of forty-eight hours, completely enclose it. As incorporation

proceeds (from twenty-four to forty-eight hours) the underlying chorionic ectoderm becomes broken and allows the host mesenchyme to grow into the graft, taking blood capillaries with it. As a result of this invasion, the differentiating parts of the implant become separated and surrounded with host mesenchyme and well supplied with blood. From an examination of six grafts there is no indication at the end of twenty-four hours' duration on the host membrane that vascularization has begun. However, after thirty-one hours, blood capillaries are found to have penetrated the implant with the invading mesenchyme. Prior to this the implant apparently lives upon nutritive material contained within its cells or else absorbs its nourishment from the host vessels through the intervening ectodermal layer.

110. Gastrulation in thermal-gradient-treated eggs of Triturus torosus. Francis G. Gilchrist, Pomona College.

Eggs of this urodele were marked on the future dorsal side with spots of Nile-blue sulphate, and were then treated while blastulae with a horizontal temperature differential of about 10°C. between opposite surfaces. Warming the dorsal side favors early but abnormal (often forked, occasionally double) dorsal invaginations. Ventral lips sometimes fail entirely. The resulting embryos typically show short neural plates, less extensive mesoderm, small archentera, and are less viable. Warming the ventral side leads to the formation of the ventral lip before the lateral lips. The resulting embryos are essentially normal. Rarely the dorsal lip is suppressed, in which case dorsolateral invaginations occur, and the result is a double monster. Lateral warming results in asymmetric gastrulation. The lateral or dorsolateral lip of the warmed side forms first. The blastopore closes as an oblique slit, due to the greater movement of the warmed ventrolateral lip. The embryos which develop have more neural-plate material and more mesoderm on the warmed side.

The experiments show the independence of the lips of the blastopore in invagination. Correlative factors apparently enter in determining the extent of involution and the forward-spreading of chorda mesoderm.

111. Diagnostic stages of metamorphosis in Amblystoma opacum and Amblystoma jeffersonianum. Madeleine P. Grant, Smith College and Radcliffe College. (Introduced by Alden B. Dawson.)

The first indications of approaching metamorphosis in Amblystoma opacum are darkening of the pigmentation of the dorsal region, sudden rounding of the truncated larval snout, and beginning atrophy of the tail fin. These changes mark an incipient metamorphic stage which precedes, by two or three days, the later metamorphic changes which begin with the first shedding of the adult skin.

The incipient metamorphic period in Amblystoma jeffersonianum is marked by a thickening and blunting of the digits, which are unusually long and slender in these larvae. In this incipient period, preceding the shedding of the first adult molt by six or seven days, changes also begin in the tail. No alterations in pigmentation occur during metamorphosis, so that coloration differences, including the bluish areas characteristic of the adult, must appear some time later.

Metamorphic stages have previously been described by the author in Amblystoma punctatum and Triturus viridescens. From studies of these four species it appears that well-defined morphological changes appear before the first adult molt and mark incipient stages. Details of the changes and the rate at which they occur vary greatly in the different species. Sufficient observations upon the thyroids have been made to suggest that the differences in the rate of change in the different species may be correlated with the degree of activity observed in these glands. The thyroid of Amblystoma jeffersonianum responded more slowly to anterior-pituitary implants and showed less activity during the metamorphic process than did the thyroid of Amblystoma opacum.

112. Skin grafting in frog tadpoles: Local specificity of skin and behavior of epidermis. Herbert W. Rand and Madeleine E. Pierce, Radcliffe College.

Unpigmented ventral skin of frog tadpoles, transplanted to the dark dorsal surface of the same tadpole or another of the same species, does not acquire characteristics of dorsal skin. In a small minority of cases (all autotransplants) it remains for months unchanged. In most cases (auto- and homoiotransplants), while to superficial observation it may appear that the graft is acquiring pigment and undergoing 'regulation' into conformity with its new environment, the fact is that its epithelium is slowly replaced in connection with an en-masse centripetal invasion of the graft territory by the surrounding pigmented epidermis. There is evidence that cells of the aggressive epithelium exert phagocytic action upon the passive epithelium of the graft.

There is no evidence that epidermal melanophores of the host advance independently of the epithelium in which they occur.

A mechanical obstacle placed at an edge of the graft blocks locally the advance of host epithelium and protects from destruction the graft epithelium of the neighboring region.

Areas were denuded of skin and covered by patches of shell membrane of hen's egg. The surrounding epidermis advanced over the membrane nearly, if not quite, as readily as over the corresponding wound surface. It did not long persist on the foreign substratum. Long after its degeneration, centripetal movement of epithelium toward the patch continued, but, no longer advancing onto the membrane, the on-coming epithelium piled up at its edge to form a thick ridge. Apparently the persisting free edge somehow occasions the continued advance of epithelium.

CYTOLOGY

Read

113. A new method used in studies on the living cells of insects. W. J. Baumgartner, University of Kansas. (Lantern; 12 min.)

Using a technic in which the living insect is fastened to a glass slide, and the testis is drawn forth slightly from the body, and the testicular follicles suspended in a lake of modified Ringer's solution, the author and a graduate student have been able to study living cells within the cysts inside the follicles.

Here the cells were in their natural surroundings under the usual pressure of the neighboring cells and bathed by the body fluids containing their foods and possible enzymes. For this reason we thing this 'intra-vitam' technic permits the study of the germ cells under the most nearly normal conditions ever attained.

The following things could be readily seen: the tridimensional nature of the follicles, the relative loci and variations in the shape and size of the cysts, the growth and division stages of the spermatogonia and first and second spermatocytes, the transformation stages of the spermatids, the aggregation of the sperms into bundles and their movement and turning in the follicles and vas deferens. Peristaltic movements in the follicles suggested muscular action. Heavily stained rodlets in fixed tissues indicated the presence of muscles.

Observation upon over 300 specimens has taught us to recognize most of the organelles seen in the cells of 'fixed materials' and has convinced us that practically all structures can be studied in living cells with proper light and lenses.

114. Seasonal cytolysis and regeneration in the anura. Roy A. Waggener, Carleton College. (Introduced by B. F. Kingsbury.) (12 min.)

A very curious cyclic change has been reported to occur, Waggener ('29), in the parenchymal cells of the parathyroids in Rana catesbeiana (Shaw). The cytoplasm was observed to break down by a kind of cytolysis and the nuclei were seen to decompose by chromatolysis and pycnosis. It was found that the entire organ would liquefy during a certain season of the year, except for a few germinal cells lining the capsule, and that the liquefied tissue was replaced by young cells which were produced from rather definite growth centers or areas. Cytolysis and regeneration appeared to progress in a sequence such that in this organ a rather large per cent of healthy tissue was always present.

More recent investigations show that cytological changes are not restricted to R. catesbeiana, but that they do occur in certain other Anura. The species which have been examined up to the present time show a cycle of changes which differ somewhat from that previously described for the American bullfrog.

Seasonal cytological changes are herein reported for tissues of R. catesbeiana other than the parathyroid and include such diverse structures as the thyroid, ventraler kiemenreste, muscle, and thymus.

115. Some cytoplasmic structures in the male reproductive cells of a pseudoscorpion (Chelanops corticis). Henry G. Nester, Indiana University. (Introduced by F. Payne.) (Lantern; 10 min.)

The chondriosomes are vesicular in the germinal epithelium. They aggregate about the Golgi cap on the nucleus, and when this moves away from the latter they change shape from spheres to cylinders. During the maturation divisions the chondriosomes and Golgi material are only approximately equally divided. In the spermatid Golgi granules are seen, for a time, on the vesicular chondriosomes. The vesicles later become disc-shaped, then spherical again. These changes in shape may be a means of regulating chemical reaction. The chondriosomes at this stage contain hollow or solid spheres and comma-shaped bodies, which I believe of Golgi origin. A thick thread finally originates from among the chondriosomes of the elongated sperm.

The Golgi material in the germinal epithelium consists of either net-like masses, units composed of lightly osmicating matrix, including heavily osmicating granules, or of dispersed granules. The Golgi cap which forms on the nuclear surface, on moving away therefrom, becomes spherical. After the chondriosomes surround it, this sphere is joined by the remaining Golgi material.

The entire mass breaks up before the maturation divisions. In the spermatid the Golgi material gives rise to the acrosome, and slightly later to spiral threads which surround the elongating nucleus. These threads persist in the mature gamete. Origin of the latter structures from the Golgi material has not been recorded previously. The acrosome, after elongating concomitantly with the nucleus, shortens and becomes club-shaped, with various parts distinguishable. The nucleus, spiral thread, the acrosome and chondriosomal thread become coiled and encysted in an oval-shaped capsule, which likewise incloses the unusually long axial filament. In the female duct the sperm becomes uncoiled.

116. A comparative study of rodent chromosomes. J. C. Cross, University of Texas. (Introduced by T. S. Painter.) (Lantern; 7 min.)

This study was undertaken in order to compare the chromosomes of as many rodents as possible with the view of determining what changes have taken place in these elements during speciation. Special attention has been given to spermatogonial chromosome number and morphology. Thirteen pure species have been studied, including Peromyscus (three species and one subspecies), squirrels (four species and one subspecies), and six other species of rats and mice. The chromosome number ranges from forty rod-like elements, more or less uniform in size, to well over sixty, including many v- and j-shaped chromosomes. All Peromyscus species and subspecies studied show forty-eight chromosomes. A discussion of the bearing of these findings on the general problem will be given.

117. The influence of x-rays upon the processes of secretion in the pancreas, observed in the living cell. G. C. Hirsch, Utrecht (Holland). (Introduced by P. W. Whiting.) (Lantern; 15 min.)

The questions were: 1) Is the pancreas cell, during the entire period of restitution, equally susceptible to x-rays? 2) Is it possible to eliminate one factor of restitution by x-rays?

The methods followed were: variation of time before and after x-radiation; observation of the living cells; vital staining; and statistical treatment.

First I found that small doses of x-rays accelerate the secretion, large doses 'kill' the cell function for a long time, while medium doses retard cell function.

Only during the first four hours after pilocarpinization is the cell susceptible to x-rays (shown by comparison of curves). The pancreas cell, therefore, behaves like a cell in division.

The causes are: 1) the development of the 'old' granules in the cell before the influence of x-rays is proceeding normally; 2) the rebuilding of primary granules is retarded; 3) the mitochondria are destroyed, but rebuilt during the following hours as a surface layer in the cell; 4) after this rebuilding new primary granules appear. All these facts cause a retardation of restitution of four hours.

Conclusion: It is probable that the retardation of restitution of primary granules is due to the destruction of the mitochondria. But a physiological connection between mitochondria and granules is still unproved.

118. The structure and reproduction of the parasome or parabasal body in trichomonad flagellates. Harold Kirby, Jr., University of California. (Lantern; 8 min.)

In connection with the contention that the parasome is the Golgi apparatus of trichomonad flagellates, its composition of chromophile and chromophobe substance, its supposed secretory activity and its supposed reproduction by division have been discussed. In Trichomonas termopsidis certain technique demonstrates a chromophile thread along one side of the parasome, but by other methods a clearly defined internal structure has been revealed. After Schaudinn's fluid or mercuric chloride, Delafield's haematoxylin stains a single row of large granules within a lightly staining matrix bordered by the sheath of the parasome. The same structure has been shown after Champy's fluid by Delafield's and by Regaud's haematoxylin, after formalin vapor by Delafield's, and by other technique. A similar structure, but with larger chromatic blocks in place of granules, has been demonstrated in Metadevescovina debilis and other Devescovininae. Sometimes in these the clearer areas are enclosed by the chromatic part. No evidence of droplets being released from the parasome has been seen in several species of trichomonad and devescovinid flagellates. Always it has a smooth, clearly marked boundary. In Trichomonas termopsidis, at the time of division, the old parasome is resorbed and two new ones, which at first are pointed posteriorly, are differentiated in connection with the blepharoplasts. Outgrowth is completed by the late anaphase. Alexeieff ('24) reported that the old parasome of Tritrichomonas augusta is resorbed. It is probable that outgrowth, and not division, is the universal method of origin of new parasomes, as well as of axostyles, in Trichomonadidae.

119. The zone of Golgi in the cartilage cells of Necturus. Alden B. Dawson, Harvard University. (Lantern; 10 min.)

In supravital preparations, the zone of Golgi appears as a homogeneous area of cytoplasm in which bodies stainable with neutral red, and filaments stainable with Janus green B are grouped. The bodies rendered visible with neutral red also appear in certain osmic impregnations as blackened granules. Since such preparations may be obtained from animals not previously treated with neutral red, these bodies are undoubtedly preexistent. Additional bodies may be formed as a result of the reaction with neutral red, and these may also react with osmic acid. The filaments which occupy the zone of Golgi are usually discontinuous and do not form a network. They react more or less specifically with osmic acid, but may be demonstrated equally well by several mitochondrial methods. In mature cells they are indistinguishable morphologically from the filamentous mitochondria of the rest of the cytoplasm. In younger cells they are readily differentiated from the rod-like and granular mitochondria. In this instance the interpretation of the osmiophilic filaments as a specific Golgi substance or as modified mitochondria would appear to depend on the individual evaluation of the criteria utilized to distinguish such cell components. The zone of Golgi usually occupies a definite area of the cell. In young flattened cells it is found near one pole of the nucleus. In the more rounded, deep-lying cells it has a paranuclear position and is always on the side away from the surface of the cartilage, except in recently divided cells, in which case it lies on the side nearest the other member of the pair.

120. Carbohydrate metabolism in relation to the mitochondria of the hepatic cell. J. McA. Kater, Washington State College. (Lantern; 15 min.)

Previous work has failed to show any correlation between the morphology of the mitochondria of the hepatic cell and carbohydrate metabolism. In the present work no attempt has been made to correlate mitochondrial morphology with the glycogen content of the liver, but rather with the rate of change of glycogen into glucose and vice versa.

In a cat that has not been fed for twenty-four hours, the mitochondria are small and inclined to be rod-shaped. They are more numerous and slightly larger near the central vein than at the periphery of the hepatic lobule. If hyperglycemia is produced in such an animal by the administration of adrenalin hydrochloride or by ether anaesthesia, the mitochondria become greatly hypertrophied and become almost entirely converted into spherical form. The effect is more pronounced in the center of the lobule than at the periphery. The production of hypoglycemia by the injection of insulin brings about exactly the same response on the part of the mitochondria as does hyperglycemia.

It seems probable that the difference between these results and those of earlier workers is traceable to the fact that a kinetic aspect of carbohydrate metabolism rather than a static one is being dealt with in the present investigation.

121. A consideration of the central-body situation in the second cleavage of Ascaris megalocephala. Lloyd C. Fogg, Washington Square College, New York University. (Introduced by Henry J. Fry.) (Lantern; 15 min.)

This study attempts to ascertain whether the central body in Ascaris is an actual body or an artifact.

The eggs are allowed to develop in 0.9 NaCl, Ringer's solution, or in distilled water. In all cases development is not affected, since cleavage continues normally. Samples of eggs from a given set are fixed in Gilson-Carnoy periodically. After staining with Heidenhain's iron haematoxylin, a period-like granule is consistently present. This granule (centriole) appears during early prophase as a double structure inclosed in a homogeneous area (centrosome) surrounded by only a trace of a radiate configuration (astral rays). Up to and during the metaphase, the centriole increases in size and takes the stain more intensely. Subsequently it gradually loses its staining capacity. It may vary in shape, but usually becomes somewhat elongate, and finally is demonstrated only with difficulty in the late telophase again as a period-like granule. It divides during the interkinetic period. The staining capacity of the centriole is different from both the rays or the chromosomes. The rays take oxychromatic stains, the chromosomes and centrioles basichromatic, but the centriole does not stain with Feulgen or with Auerbach's acid-fuchsin methyl green. This history agrees in essential details with that of earlier workers.

Recent suggestions that the centriole could be mistaken for random granules do not hold in Ascaris, for no random granules similar in size or staining capacity are to be found. That the centriole may be the focal point of rays is not evident with this fixative and stain.

122. Nuclear behavior during the formation and secretion of the silk in Bombyx mori. Hope Hibbard, Oberlin College. (Lantern; 10 min.)

The silk gland of Bombyx presents a single, unrepeated cycle of development. From its condition several days before the larva hatches from the egg until well into the pupal stage the cells may be followed. The nuclei, at first unbranched, grow progressively more and more branched without fragmenting, spreading throughout the cytoplasm until the cell is nearly all nucleus. After the lumen of the gland has been emptied during the spinning of the cocoon, the cells settle closer together and practically no cytoplasm is left. Even the duct leading from the storage reservoir of the gland has a highly branched nucleus. The possibility suggests itself that this material (chromatin), later absorbed during the pupal period, may serve as stored material to be made over into new tissues during the metamorphosis, much as the stored fat is so used.

As to the relation of this nucleus to the formation of the secretion, there is no emission of chromatin into the cytoplasm at any time. The small microsomes (of oxychromatin, according to Heidenhain) are found to be of chromatin material by the nucleal reaction (Feulgen); the macrosomes (basichromatin of Heidenhain), or nucleoli, stain under no circumstances with the nucleal reaction. The author is unable to observe the passage of solid nucleoli through the nuclear membrane. It is true that granules in the cytoplasm near the nucleus stain like nucleoli by the Altmann method, but they are thought to be mitochondrial in character.

By demonstration

123. The occurrence of both anatomical (hepatic) and functional (portal) lobules in the liver of the seal. L. B. Arey, Northwestern University Medical School.

124. Chromosomes of three species of Mantidae (Orthoptera). Robert L. King, State University of Iowa.

There are thirteen first spermatocyte chromosomes in Tenodera sinensis, Stagmomantis carolina, and Mantis religiosa. Twelve of the chromosomes in each case are tetrads made up of two homomorphic atelomitic dyads. The thirteenth chromosome is a tripartite element (hexad) in each of the species. In Tenodera sinensis and Mantis religiosa it consists of three large atelomitic dyads joined end to end with the smallest element in the middle. In Stagmomantis carolina the tripartite element consists of two large atelomitic dyads with a spherical dyad between them. The tripartite element is interpreted as a sex-determining mechanism of the constitution X_a—Y—X_b.

The history of the chromosomes has been followed in Tenodera sinensis. There are twenty-seven atelomitic chromosomes in the spermatogonial metaphases, two of which are conspicuously larger than the others. These two and one other large chromosome fail to pass directly to the pole of the cell in telophase, but lag behind the others and form a separate vesicle which eventually fuses with the telophase nucleus. The history of these chromosomes may be traced through the prophases of the first spermatocyte to the metaphase where they appear as the tripartite X_a—Y—X_b.

In the first maturation division each of the twelve tetrads divides, the tripartite element (hexad) divides, with X_a—X_b going to one pole and Y to the other. This gives rise to second spermatocytes with $12+X_a+X_b=14$ chromosomes and those with $12+Y=13$ chromosomes.

There are twenty-eight chromosomes in the somatic cells of the female, of which four are conspicuously larger than the others. The constitution of the female is interpreted as $24+2X_a+2X_b$, that of the male as $24+X_a+X_b+Y$.

125. A cytological study on the spinal-ganglion cells of the rat. H. W. Beams, State University of Iowa. (Introduced by J. H. Bodine.)

The Golgi apparatus, mitochondria, vacuome, canalicular apparatus (trophospongium of Holmgren), and Nissl bodies have been demonstrated in the spinal-ganglion cells of the rat following appropriate techniques.

It is suggested that the reticular apparatus of Golgi, short filamentous and granular mitochondria, vacuoles (vacuome), and irregular-shaped Nissl bodies are discrete in the spinal-ganglion cells of the rat. The application of Mann's, Champy's, and 2 per cent osmic-acid solutions to tissue previously stained intravitally with neutral red, under direct microscopic observation, was not observed to stimulate coalescence of the neutral-red bodies. Only in those cases where the vitally stained ganglion cells were permitted to stand in sealed preparations for considerable time were the neutral-red bodies observed to fuse.

The canalicular apparatus of Bensley and Cowdry and the trophospongium of Holmgren ('14) are thought to represent the negative image of the Golgi apparatus. However, no direct connection of the canalicular apparatus to the surface of the cell was observed. In some cases the spinal-ganglion cells, following treatment with Mann-Kopsch (Weigl) technique, show besides the impregnated Golgi apparatus a number of clear spaces. If the recent view is correct that the Nissl substance actually exists in the spinal-ganglion cell in a diffuse condition and upon fixation it may be coagulated into irregular flakes (Addison, '29), it seems possible that the appearance of these clear spaces may in some way be associated with the coagulation phenomena of fixation.

126. Demonstration of human X-Y sex chromosomes. T. S. Painter, University of Texas.

For more than a year the author has been studying the testicular tissue of a Mongolian idiot with a view to ascertaining if this form of idiocy could be conditioned by chromosomal abnormalities. Up to the present no evidence for this has been obtained. Incident to the study, however, has been the examination of a great deal of well-fixed material in which there are many first-spermatocyte spindles showing the X-Y sex complex. In view of discussion which has been going on about the existence of such sex chromosomes in man, and especially the denial of the presence of the Y, by certain cytologists, a demonstration of my new material seems desirable.

127. A demonstration of the nuclear changes during the growth and secretion of the silk gland of Bombyx mori. Hope Hibbard, Oberlin College.

This is in partial illustration of the paper on the cytological changes during secretion in the silkworm.

128. A demonstration of the intimate penetration of tracheal tubules into the deep cytoplasm of gland cells. Hope Hibbard, Oberlin College.

This recalls a similar case of intracellular penetration of tracheal tubules into the nerve cells of the grasshopper, reported at the meetings in Des Moines by Ross and Tassell.

By title

129. Studies on the gastric epithelium of grasshoppers. B. H. Woodruff, Western Reserve University. (Introduced by J. P. Visscher.)

Observations were made on miscellaneous specimens in both the fixed and the fresh state mounted usually in normal saline. The epithelium is cuboidal, except in the midgut and the attached gastric caeca where the cells are columnar. The cells of the gastric caeca may or may not be ciliated. The distal ends of these cells are often more densely granular than the proximal ends and swell with secretions until the tips of the cells are freed into the lumen to mix with the food. Cells worn out in this manner are replaced from crypts of regenerative cells. The mitochondria are minute granules arranged in the basal half or third of the cell, resulting in a striated appearance of the cytoplasm.

In many preparations very large vacuoles of two sorts appear. Those of one type are closely associated with the nucleus, lying at either or both ends of it, sometimes compressing it. Other methods show that these represent the dissolved Golgi material.

The other type of vacuole is usually between the nucleus and the lumen end of the cell, often enormous, spreading across the distal parts of two or more cells and containing dark granular masses. This type of vacuole results from the intracellular development of gregarines, the adults being found in the lumen. Such vacuoles are lacking in specimens hatched and grown in the laboratory.

130. Effects of experimental inanition and recovery on the blood cells of the salamander, Triturus viridescens. H. E. Jordan, University of Virginia.

After a prolonged period of starvation, to the point of imminent death, large numbers of erythrocytes show a greatly altered nucleocytoplasmic relationship and a very atypical condition of the nuclear structure. The lymphoid hemoblast at various stages of differentiation shows comparable alterations. Individuals starved for four months have barely enough blood for a single small smear. Eosinophils are practically lacking, and the basophilic granulocytes have a much altered foamy cytoplasm. The thrombocytes and neutrophils appear about normal in number and structure, except that a certain number of thrombocytes contain a variable amount of black granules.

In some of the specimens approximately 50 per cent of the red cells are abnormal. In extreme conditions the nucleus occupies almost the entire cell. The nucleus is characterized by an extremely fine-meshed delicate reticulum.

These atypical cells appear identical with some of those described by Nigrelli ('29) in specimens of the same species infected with trypanosomes (Anat. Rec., vol. 43, p. 3). No evidence of infection was found in our material. The abnormal erythrocytes seem entirely the result of starvation, the modification apparently representing in part an endosmotic nuclear effect of a rarefied cytoplasm. As in Nigrelli's collection after liver feeding, the condition in our material disappeared after feeding with earthworms. At the end of the second week the blood was essentially normal, except for large numbers of young and dividing cells and an increased number of thrombocytes with black granules.

131. The pigment content of the liver cells of urodeles. H. E. Jordan, University of Virginia.

The liver of certain urodeles (Triturus, Amphiuma) differs structurally from that of anurans in that many of the tubules are composed of only two rows of cells. In Amphiuma approximately half of the hepatic parenchyma contains a voluminous content of finely granular brownish pigment. The pigmented cells in sections occur in groups of from two or three to many, corresponding with transverse or oblique sections of tubules. Hepatic cells contain either a large amount or none of the pigment. Appearances suggest a division of function between pigment-handling tubules and the usual type of hepatic tubule. Only a small number of the cells in any section, and in these only a variable number of larger granules, give the Prussian-blue reaction for iron.

Livers of Triturus viridescens vary greatly in amount of pigment. When only a small amount occurs, it is confined to macrophages within sinusoids; where much appears, it occurs also in Kupffer cells and certain hepatic cells. The spleen contains many macrophages with ingested erythrocyte fragments converted in part into hemosiderin granules and iron-free pigment granules. These cells pass into the liver sinusoids, where, following disruption, they deposit their mixed content. This is phagocytosed by Kupffer cells and then transferred to liver cells. Here it occurs for the most part as minute spherical iron-free granules. Apparently identical granules occur in bile from the gall bladder.

132. The vacuome of the hypotrichous ciliate, Stylonychia. R. P. Hall, New York University.

In Stylonychia pustulata small endoplasmic globules (vacuome) are stained vitally with neutral red in dilute solutions. The vacuome may be differentiated from mitochondria by staining vitally with a mixture of neutral red and Janus green. In addition, the vacuome may be stained with neutral red and subsequently osmicated in sealed-slide preparations under direct observation; the neutral-red globules are gradually blackened. Globules similar in size and distribution are blackened by osmic and silver impregnation. In Golgi's ('20) gold-chloride method globules of similar size are blackened, as are also the contents of food vacuoles; but the most striking effect in such preparations is the appearance of extremely numerous precipitation granules (?) throughout the cytoplasm and even in the cirri. These are not mitochondria or any other inclusions which have been demonstrated vitally in Stylonychia. In addition, most specimens showed a decided clumping of the endoplasmic globules—an effect not noticeable in osmic, silver, or neutral-red preparations.

Food vacuoles are formed by gradual constriction from the base of the gullet. Adherence of neutral-red globules to developing food vacuoles was not noted, nor was penetration of food vacuoles by the globules observed.

133. On the vacuome of the phytomonad flagellate, Chlamydomonas. R. P. Hall and R. F. Nigrelli, New York University.

A vacuome consisting of small endoplasmic globules has been demonstrated in Chlamydomonas sp. These inclusions may be seen in the living unstained organism, and may be stained vitally with neutral red in dilute solutions. In

preparations stained vitally with a mixture of neutral red and Janus green the vacuome is differentiated from mitochondria. The globular inclusions have been stained vitally with neutral red and then blackened gradually with osmic acid under direct observation. Similar globular inclusions have been demonstrated by the Mann-Kopsch (Weigl) and Kolatchev methods of osmic impregnation and by the Da Fano silver method. Similar globules were also colored blue in preparations stained with iodin-potassium iodide solution, indicating the presence of starch.

134. On the vacuome and the neutral-red reaction in Paramecium caudatum. F. W. Dunihue, New York University. (Introduced by R. P. Hall.)

Globular inclusions were demonstrated vitally with neutral red and also by osmic impregnation in Paramecium caudatum. A mixture of neutral red and Janus green was used to differentiate them from mitochondria. Cytoplasmic distribution of the globules is rather uniform, with the exception that they seem more numerous in the region immediately surrounding the forming food vacuole. In osmicated sections the distribution seemed approximately the same per unit volume. The globules (vacuome) are closely packed on the food vacuole during formation, but penetration of the vacuole by the globules, as recently reported by Volkonsky, was not observed.

In the neutral-red reaction stained globules are first seen in the posterior region, shortly afterward in the anterior. The course of the vacuoles during digestion and their approximate pH have been checked. The neutral-red reaction in conjugating and dividing stages, which were not feeding, was essentially similar to that in vegetative stages. Starvation greatly increased susceptibility of Paramecium to neutral red, diffuse staining occurring immediately in very weak concentrations which stained only the inclusions of normal organisms.

The vacuome in Paramecium seems to be essentially similar to that described for other groups of Protozoa. Distribution of the globules does not seem to support the view of Park that there is a well-defined "osmic acid differential in Paramecium." There seems to be no evidence for the existence of a complete 'digestive system' as suggested by Koehring ('30).

135. On the relation of the vacuome to metabolism in Euglena, Chlamydomonas, and Chlorella. R. P. Hall and F. W. Dunihue, New York University.

Euglena gracilis, Euglena anabaena, Chlamydomonas sp., and Chlorella sp. show globular inclusions which are stained blue to black in iodin-potassium iodide solution. In Euglena these globules which react with iodin are distinct from the paramylum bodies. Globules similar in size and distribution are stainable vitally with neutral red. In preparations stained vitally with neutral red and subsequently treated with iodin, the neutral-red globules (vacuome) gradually develop a definite blue to black color. As pointed out by Pringsheim ('27) for Polytoma, the reaction of the starch-containing inclusions is black in excess of iodin, and blue otherwise. As indicated by the iodin test for starch, therefore, it seems probable that the vacuome serves in the storage of starch in the holophytic Protozoa, Euglena and Chlamydomonas, and in the alga Chlorella. Guilliermond and other workers have previously demonstrated that the vacuome plays a similar rôle in metabolism in many different types of plants.

136. The so-called 'segregation apparatus' of the erythrocyte of frog and Necturus blood. H. W. Beams, State University of Iowa. (Introduced by J. H. Bodine.)

The writer's observations indicate that the so-called 'segregation apparatus' of the erythrocyte of the adult frog (Rana pipiens), which has been carefully described by Jordan ('25), DeRoo and Ufford ('30) (in leopard frog), cannot be directly homologized with the 'segregation apparatus' of the erythrocyte of Necturus recently studied by Dawson ('28). The writer's evidence is in agreement with Dawson's ('29) that the 'segregation apparatus' of the erythrocyte of Necturus is probably a normal preformed structure. However, such does not appear to be the case in the erythrocyte of the frog because: 1) the segregation apparatus does not appear in favorable preparations until twenty to thirty minutes after exposure to the neutral red, although it may appear somewhat earlier following exposure to Nile-blue sulphate; 2) it is rarely stained in smear preparations with Wright's and Giemsa's stains; 3) at about the same time that it takes the 'segregation apparatus' in the frog erythrocyte to appear, a secondary group of neutral-red bodies (induced bodies of Dawson, '29) are seen to appear in the erythrocyte of Necturus.

From this evidence it is suggested that the 'segregation apparatus' of the erythrocyte of frog blood (Rana pipiens) and the induced bodies of the erythrocyte of Necturus are comparable to the condition described by Cunningham ('30) for white blood cells of man in "that the formation of vacuoles in normal cells results from the accumulation of waste products, the abnormal environment being the stimulus."

137. The release of follicular colloid from the thyroid of Amblystoma larvae following heteroplastic anterior-pituitary implants. Madeleine P. Grant, Smith College and Radcliffe College. (Introduced by Alden B. Dawson.)

Studies of the urodele thyroid during different stages of activity were made with Champy fixation and hematoxylin and Mallory's connective-tissue stains. The details observed were seen only in material fixed in Champy. Zenker and Helly fixatives and modifications of these, including Zenker with osmic acid, failed to bring out such structures. Thyroids of normal Amblystoma jeffersonianum larvae presenting no incipient metamorphic changes contained numerous follicles distended with stored secretion and lined only with low cuboidal cells. Occasional follicles contained a few cells which were columnar in shape and filled with large non-staining vacuoles. Occasional colloid droplets were seen in both the vacuolated and the non-vacuolated cells.

Implants of frog anterior pituitary were made into the peritoneal cavities of similar larvae. These received daily implants for one, two, three, four, five, or six days, and were killed twenty-four hours following the last implant. The glands from individuals which had received one, two, and three implants showed no marked changes from the normal picture, except for slight but definite increases in the number of vacuolated cells. After four successive implants the number of vacuolated cells had greatly increased, while the size of their vacuoles had decreased. The most striking change at this stage was the marked decrease in the amount of intrafollicular colloid accompanied by a simultaneous increase

in the amount of intracellular colloid. Following five or six daily implants, the follicles were completely emptied and all the thyroid cells were saturated with colloid which appeared in the form of an 'emulsion' in the cytoplasm.

138. The release of follicular colloid from the thyroids of Amblystoma larvae during diagnostic stages of metamorphosis. Madeleine P. Grant, Smith Colleg and Radcliffe College. (Introduced by Alden B. Dawson.)

Experimental work upon urodele thyroids which have been stimulated to release the follicular colloid by anterior-pituitary implants has offered material which could be studied at precise time intervals. The process therefore could be followed from the initial step through complete emptying of the follicles. As has been elsewhere reported, the process began with an increase in the number of vacuolated cells. Later, with reduction in the quantity of stored colloid, there appeared an increase in intracellular colloid. With these pictures in mind, an analysis of the histology of the thyroids of Amblystoma opacum and Amblystoma jeffersonianum during diagnostic stages of metamorphosis has been made.

From observations upon the thyroids of these urodeles, the following conclusions have been drawn: 1) Increased activity in the gland began during the incipient metamorphic period, and colloid release slowly progressed throughout the transformation stages. 2) Reduction of follicular colloid, although considerable, was never so complete as that obtained in the experimental series, and therefore the intracellular colloid was never so great. 3) The process of colloid release during metamorphosis showed the same series of histological pictures as that seen in the experimental studies and likewise gave evidence of transcellular export of stored colloid. The process occurred much more slowly and was never complete, so that, without the exaggerated pictures of the experimental studies, interpretation would have been less convincing.

139. The services of the beaker cells in the skin of Amphibia. J. V. Shankweiler, Cornell University. (Introduced by Secretary of Section F.)

Literature upon the beaker cells disclosed a difference of opinion regarding the rôle played by these cells in the skin of Amphibia. A combined experimental and structural study points clearly to the conclusion that the important function of these cells is that of facilitating the molting process.

140. Some results of the application of Feulgen's nuclear reaction to the early stages in Odonate oogenesis. Arthur D. Whedon, North Dakota Agricultural College.

For years cytologists have been interested in the peculiar 'double nucleoli' of the oocytes of Odonate larvae. McGill ('05) attempted analysis by differential staining, and concluded that these nucleoli arise by "condensation of the basiphile chromatin-spireme around the oxyphile nucleolus"—a process comparable to synapsis. Hogben ('20) disagreed decidedly with this view, and accounted for the double nucleolus as resulting from "a process of internal differentiation."

Recently Feulgen's nuclear reaction has been accepted as a quite universal test for chromatin, and has been applied to various materials by Koch, Ludford, Gresson, and others.

The writer first saw these double nucleoli in the oocytes of Sympetrum, Libellula, and Anax about 1918, and recently has applied the Feulgen reaction in the hope of determining their structure. Ovaries were fixed in Bouin's fluid, sublimate, and Carnoy's reagents. Controls were run without hydrolyzing, and sections were stained with iron haematoxylin. In no case did any portion of oocytes containing double nucleoli react to the chromatin test, though the earlier stages of eggs and all follicle cells responded strongly. This is in accord with Gresson on Tenthredinids ('29).

Thus the decision seems inevitable that there is a marked change in the thymus-nucleic acid of chromatin during certain stages of oogenesis and that Feulgen's reaction is effective only under limited conditions of aldehyde formation from nucleic-acid complexes. It is improbable that chromatin should entirely disappear from the developing egg at any stage.

141. Central bodies in reciprocal hybrids of Echinarachnius and Arbacia. Henry J. Fry and Jacob Katz, Washington Square College, New York University.

The mitotic figures of fertilized Echinarachnius eggs when compared with those of Arbacia show distinct differences concerning, 1) the size and physical structure of central bodies (centrosomes), asters, and spindles; 2) the ratios between the volumes of cells, asters, and central bodies; 3) the time schedule of the mitotic changes.

Whether Echinarachnius eggs are fertilized by Echinarachnius sperm or by those of Arbacia, in both cases the mitotic phenomena are identical and are typical of Echinarachnius. Similarly, Arbacia eggs have mitotic figures in all respects typical of Arbacia whether they are activated by Arbacia or by Echinarachnius sperm.

The current hypothesis suggests that a central body may be introduced into the egg by the sperm. It is assumed to play some rôle concerning the mitotic figure, at least acting as the formative focus of the asters.

The present study of reciprocal hybrids shows that, no matter what structures, whether visible or invisible, may be introduced by the sperm of either species, all observable mitotic phenomena—including structure, volumetric relations, and time schedule of the central bodies—are determined solely by the maternal cytoplasm. The evidence is unfavorable to the classic view. It is in harmony with the assumption that central bodies of echinoderms are not individualized structures in the living egg, but are only coagulation products of the focal area of the astral rays, the detailed configuration of which is determined by the egg's cytoplasm.

142. Central bodies during successive cleavages in Echinarachnius eggs. Henry J. Fry and Malvina Schweizer, Washington Square College, New York University.

During early cleavages of Echinarachnius eggs (fixed in Bouin's fluid) the central body of prophase asters is a granular centrosome undelimited from the ray area; that of metaphase figures is pleuricorpuscular and sharply delimited; anaphase asters have a giant vacuolar centrosphere.

About the sixteen-cell stage these types disappear or are modified. A minute period-like centriole is found in certain percentages of prophase and metaphase asters. As cleavage progresses the percentages vary in an orderly manner, but during any one cleavage the per cent of prophase asters having centrioles differs from that in metaphase asters. Furthermore, the prophase centrioles differ in physical structure from the metaphase ones. Meanwhile, the vacuolar centrosphere of early anaphase asters becomes a condensed pleuricorpuscular body, and finally a granular undelimited area. During early gastrulation asters completely disappear together with the central bodies.

These modifications are probably explained by the gradual reduction in astral size and by marked changes in spindle shape which affect astral shape. Fixation under these various conditions produces different 'central-body' types, depending on the detailed ray configuration at the astral center. These radical modifications of central-body behavior are unfavorable to the idea that the echinoderm central body is an individualized structure like a chromosome or a plastid.

143. Asters in fertilized Chaetopterus eggs. Sophye Mohel and Henry J. Fry, Washington Square College, New York University.

Fertilized Chaetopterus eggs, fixed in Bouin's fluid, were studied from fertilization to first cleavage. Like most reagents, Bouin's fails to fix the astral centers of Chaetopterus eggs, leaving them 'empty.' Hence no study was made of central bodies.

Measurements of metaphase asters and spindles of first polar-body figures, compared with those of the second, show an exact ratio of 2:1; not 1.6:1 as indicated by Mead's ('98) illustrations. This ratio may be related to a similar ratio between the diploid and the haploid chromosome groups.

The sperm aster does not divide to form the first-cleavage figure as described by Mead; it disintegrates and the cleavage amphiaster arises as a new structure. Hence the mode of origin of the first-cleavage figure falls under the same category as that of later ones. Future study of various species will probably demonstrate that an aster seldom, if ever, actually divides to form an amphiaster.

Spindle elongation is negligible. It cannot account for the movement of the chromosomes—a possibility suggested by Bělař in other species.

It is doubtful if the cleavage asters are directly concerned with the segmentation of the cell, as is generally assumed. In both sperm asters and cleavage figures the asters pass through a cycle of similar structural changes, becoming distinct only when nuclei or chromosomes begin to change position, and immediately fading when the movement is completed, regardless of whether segmentation is imminent or not.

144. Comments on cell nomenclature according to Gatenby.[1] Hope Hibbard, Oberlin College.

Professor Gatenby's definition of the Golgi apparatus intensifies and perpetuates the confusion that reigns in the literature. It allows the inclusion of osmiophile fat-droplets; of argentophile mitochondria, as in the perch egg; of argentophile and osmiophile vacuoles vitally stainable with neutral red; all of which cases Professor Gatenby would exclude. As Owens and Bensley[2] pointed

out before the publication of Gatenby's paper, the osmic-acid reaction is in no sense specific, but can be shifted at will from one cellular component to another.

Furthermore, one wonders by what authority the statement is made that "The words 'Golgi apparatus' were understood by Golgi to mean . . . ." when Golgi's own writings never included the term 'Golgi apparatus' and his 'apparato reticolare interno' was a specific observation in nerve cells.

The question of the nature of the bodies in the very special case of male germ cells, variously called dictyosomes, lepidosomes, ergastoblasts, etc., and which Professor Gatenby understands to be the osmiophilic portion of the Golgi apparatus, would seem to be best approached by histochemical studies. These bodies give both protein and lipoidal reactions, as do mitochondria; they stain vitally with Janus green, as do mitochondria; none of these is true of the 'apparato reticolare interno' of Golgi. Perhaps these, then, are more nearly related to the chondriome than to the 'apparato reticolare interno'—a relation of which Parat[3] takes note in calling them the 'active' chondriome.

If the name Golgi apparatus cannot be limited to one agreed type of cell element, it would be in the interest of scientific accuracy to drop it from general usage.

[1] Jour. Roy. Micr. Soc., 1930, vol. 50. [2] Am. Jour. Anat., 1929, vol. 44. [3] Arch. d'Anat. Micr., 1929, vol. 24.

PROTOZOOLOGY

Read

145. Paramecium infusion histories. II. a) Methods of concentration and, b) some causes of aggregation. Edgar P. Jones, University of Pittsburgh. (Introduced by Robert T. Hance.) (10 min.)

Part I. Paramecium multimicronucleatum and rotifers may be made to concentrate at the surface of a culture by adding to it at least an equal amount of hay infusion not more than four days old. The animals so banded may be further concentrated by collecting them with fine pipettes, placing them in concentration tubes, shaking, and pouring off the infusion from the aggregation which settles to the bottom of the tube.

Part II. A study of the physicochemical causes of such aggregations is made. Excretion products, hydrogen-ion concentration, and carbon dioxide-oxygen balance are considered as factors which might bring about the banding.

The experimental evidence strongly suggests that the animals are responding to the shifting carbon dioxide-oxygen balance.

It is pointed out that this phenomenon may have been incorrectly interpreted as geotropism.

146. Is Paramecium geotropic? Edgar P. Jones, University of Pittsburgh. (Introduced by Robert T. Hance.) (5 min.)

This paper questions whether the so-called geotropism of Paramecium is not in fact a 'trial and error' search by the organism for the region of optimum carbon dioxide-oxygen balance. The literature is reviewed chiefly for the purpose of demonstrating that Paramecium is irregular in exhibiting the so-called negative geotropism.

147. Studies on the contractile vacuole of Blepharisma undulans. Imogene Moore, Yale University. (Introduced by L. L. Woodruff.) (15 min.)

The normal vacuole and anus and the regeneration of these structures have been studied in pedigreed animals. The normal vacuole is not a permanent cell organ in Blepharisma, but a system of temporary, potentially independent fluid vacuoles. The typical arrangement of these components is a posterior primary vacuole with one or more secondary vacuoles around its anterior border. The secondary vacuoles typically do not empty into the primary, but instead, as the latter empties, move into its place and, if fusion has not already occurred, now coalesce to form the next primary vacuole in the sequence. The fate of any component of the system depends upon prevailing environmental conditions within the cell. The membrane of each vacuole disappears completely at systole. Excretion granules have not been found, but the 'de novo' formation of a vacuole has been observed. In operated animals the earliest vacuoles to empty are formed by coalescence of smaller vacuoles which first appeared at either contiguous or isolated points along the cut surface. The typical arrangement of vacuoles is unnecessary for the effective extrusion of their contents and comes about only secondarily as regeneration proceeds. Both food and fluid vacuoles empty through a temporary pore which may form when their membranes are in contact with a region of thin, undifferentiated ectoplasm. Such a region is constituted by the 'anus' in normal animals and by the constantly diminishing area of injury in regenerating animals. In fact, the regenerated 'anal spot' is the permanent vestige of the injured area.

148. The origin of the several genera of Opalinidae. Maynard M. Metcalf, Johns Hopkins University. (Lantern; 12 min.)

Each genus shows in its life history one or several larval stages, each corresponding to the adults of another genus. Drawings of these larvae are shown for each genus and several subgenera. Their phylogenetic significance is so evident as to need little discussion beyond calling attention to the fact that the evolution of these parasites is due mainly to factors within the organisms themselves, rather than to environmental influence.

By demonstration

149. The parabasal apparatus and cytoplasmic inclusions of Trichonympha from Termopsis. Harold Kirby, Jr., University of California.

Numerous cord-shaped parasomes are present in T. campanula and T. sphaerica. Each has a sheath staining more deeply along one side and an inner, less stainable part often subdivided as if vacuolated. Generally some parasomes lie against the nuclear membrane, apparently providing suspension. Cytoplasmic inclusions of T. campanula are: peripheral granules, in rows beneath the ridges in the outermost ectoplasmic layer, often absent; deeper ectoplasmic granules, neutral-red staining, in the fluid middle ectoplasmic layer, variable in number or absent; minute, Lugol-staining granules, filling entire endoplasm; chondriosomes, stout rods, or granules, abundant in postnuclear endoplasm; irregular masses or groups of granules, suggestive of Golgi elements in Aggregata and Stylorhynchus, staining by mitochondrial methods, visible unstained, in prenuclear endoplasm,

especially peripheral; ingested wood, cellulose, or organisms; spherules and granules, products of digestion. In T. sphaerica are characteristic spherules absent in T. campanula. Five parasitic or symbiotic micro-organisms in T. campanula are: Sphaerita; a small, rod-like organism, varying greatly in length, often about 5 μ, usually present in abundance around or anterior to the nucleus; irregular clusters of small granules, probably bacteria, often present in T. campanula from Termopsis laticeps; fusiform bodies, about 20 μ long, pointed sharply at both ends, infrequent; small, deeply staining peg-shaped bodies, pointed at one end, with a clear area near the rounded end, about 3 μ long, infrequent. Larger rods or chains of bodies shaped like the last are probably stages in its development. Additional observations on the general structure of Trichonympha are presented.

By title

150. Studies on the plates of the fresh-water Ceratium, the so-called Ceratium hirundinella. C. T. Hurst and D. R. Strong, Western State College of Colorado. (Introduced by the Secretary of the Society.)

Exclusive of the girdle, the theca of all the members of the genus Ceratium (Protozoa, Dinoflagellata) are made up of four series of plates, the apical, the precingular, the postcingular, and the antapical series. There is considerable disagreement in the literature regarding the number of plates in these series. This is especially true of the first two above-mentioned series. This report deals only with the apical series of plates.

Kofoid ('07, Zool. Anzeiger, Bd. 32, no. 7, p. 177 ff.) describes four apical plates in all the subgenera of Ceratium. Jörgensen ('11, Die Ceratien, p. 3; Int. Rev. der ges. Hydrobiol. u. Hydrograph., Bd. 4, Biol. Sup., Heft 1) also says there are four such plates. According to Entz ('27, Archiv für Protistenkunde, Bd. 58, p. 350, etc.), this apical series of plates contains only three plates in the form he calls C. hirundinella, one of the common fresh-water species of this genus. He shows the first apical plate as forming the left half of the apical horn, the second forming the posterior right quarter, and the third the anterior right quarter. We find that in a form identical in every way with Entz's C. hirundinella there are, on the contrary and in agreement with the other authors, four apical plates. These are difficult to demonstrate and require much time to resolve them completely. To separate these plates we use a trade-marked preparation called "Clorox," a bleaching and deodorizing fluid. At first the horn separates into three plates. However, under continued observation, the third plate always splits into two. The cement is clearly seen to come out of the suture as fine particles.

151. Membranelles of Stenosemella nivalis. Arthur S. Campbell, Saint Mary's College.

The erect peristomial margin carries an adoral wreath which is a more or less flat spiral generated from the acentric mouth. The twenty-four membranelles are relatively large, long, and narrow, forming petal-like blades. They are inserted crosswise, obliquely, on the peristomial margin. Each is made up of a line of about eight adjacent lamelles and each of the latter consists of a dense rodlet and a thin, wide hyaline sheet. In cross-section the sickle-shaped lamelle

presents the following: *a*) a surrounding pellicular membrane; *b*) the ground-substance in which are, 1) six fibrils in the blunt, outer, dense region and, 2) a single fibril in the hyaline matter, on the convex side. In the dense substance, at the blunt end, surrounded by the six fibrils are varicose-like vacuoles. Basal granules connect, indirectly, these fibrils to the adoral fiber, and this is attached to the granular neuromotorium. The above structure is in essential agreement with that given by Entz, Jr. ('29), for Petalotricha ampulla. The fact that these organelles are dissimilar to the membranelles of Entodiniomorpha adds justification to the subordinal status claimed for Tintinnoinea by Kofoid and Campbell ('29).

152. Further studies of the sequence of Protozoa in five plant infusions. W. Byers Unger, Dartmouth College.

Similar experiments reported by the author in 1929 (Anat. Rec., vol. 44, p. 248) have been continued. Studies of infusions originating from plants grown in 1930 revealed some variations in the protozoan sequence when compared with former results.

During the first fifty days of the 1930 observations, protozoa appeared in infusions in the following order:

1. In Aspidium marginale—Bodo sp., Colpoda sp., Amoeba sp., Bodo fusiformis, Astasia sp. On the fiftieth day Colpoda sp. and Amoeba sp. were present.

2. In Aster sp.—Bodo sp., Enchelys pupa, Colpoda sp., Colpoda inflata, Bodo globosus, Amoeba sp. On the fiftieth day Colpoda sp., Colpoda inflata, and Bodo globosus were present.

3. In Hydrangea sp.—Bodo sp., Colpoda sp., Bodo globosus, Astasia sp. The only protozoa present at the end of fifty days were Bodo globosus and Colpoda sp.

4. In Myrica asplenifolia—Bodo sp., Colpoda sp., Bodo mutabilis, Colpoda inflata, Enchelys pupa, Amoeba sp. On the fiftieth day the last two genera were present.

5. In Trifolium pratense—Bodo sp., Colpoda sp., Colpidium sp., Colpoda inflata, Bodo globosus, Amoeba sp. On the fiftieth day the protozoan population of the culture was represented by Colpoda sp., Colpidium sp., and Amoeba sp.

153. A mutation of Chilodon uncinatus produced by ultraviolet radiation. Mary Stuart MacDougall, Agnes Scott College.

In cultures of Chilodon uncinatus, twice exposed to ultraviolet radiation, a tailed form arose which has the same chromosome number as the form from which it came, namely, a diploid number of four and a haploid number of two. The shape of the body is distinctly changed, being more pointed, and there is a change in ciliation. The new organelle disappears and is reformed during division, and also after the members of a pair separate in the conjugation process.

Results seem to indicate that the strain obtained by the isolation of a large exconjugant, selected for its size and length of tail, is homozygous, for after twnety-four conjugation epidemics there is no sign of reversion to normal. In another strain, in which the tail is shorter, five out of seventy-five pairs have reverted to normal. These normals have never shown any sign of an appendage. Some results have been obtained in crossing back the normal to the tailed form, but more data will have to be obtained before the significance of these results can be determined.

154. On the morphology and binary fission of Heteronema acus. J. B. Loefer, New York University. (Introduced by R. P. Hall.)

The nuclear structure of Heteronema was observed in both resting and mitotic stages. The endosome may be either single or composed of as many as seven fragments, which are often vacuolated. Small chromatin granules surround the endosome in interphase. These unite to form thread-like chromosomes which separate after splitting to lie parallel to the divided endosomes, accompanying the endosomes as they draw apart during anaphases. No rhizoplasts were observed. One of the two flagella passes to each daughter cell in fission, while two new flagella grow out from blepharoplasts. The 'staborgan' consists of a falcate and two parallel rods; the anterior ends of the latter always touch the gullet, and sometimes extend to the cytostome. The free end of the falcate rod approximates the rim of the cytostome only in feeding, being located more posteriad in the resting condition. The posterior ends of the parallel rods vary in position with respect to the reservoir during metaboly. The flagella apparently pass below the falcate rod into the gullet, which lies above the two parallel rods. Some specimens show the falcate rod definitely opening the cytostome, and in one case the gullet contained a food body in process of ingestion. During division the old 'staborgan' (pharyngeal-rod apparatus) disappears posteriad, and two new sets arise de novo as described by Rhodes ('26).

155. Cultural reactions of Chlamydomonas sp. R. F. Nigrelli, New York University. (Introduced by R. P. Hall.)

Bacteria-free cultures of Chlamydomonas sp. were obtained by the dilution and plating method. "Difco" dehydrated culture media were used. Heavy growth was obtained in tryptophane broth (pH 8.0), lactose broth (7.5), dextrose broth (7.3); good growth in sucrose broth (7.7), starch agar (8.0), maltose broth (7.5); fair growth in nitrate broth (8.0), nutrient gelatin (6.5), nutrient agar (7.3), nutrient broth (6.6), purple lactose agar (6.5). Good growth was obtained in darkness in the following: tryptophane broth, dextrose agar, and dextrose broth. In investigating oxygen relationships, tubes were inoculated, inverted, and the air displaced with hydrogen. The inverted tubes were then placed in a Novy jar filled with hydrogen. In light, good growth occurred in forty-eight hours in dextrose, Andrade-lactose, Andrade-dextrose, Russell's double-sugar, and starch agar shakes. All tubes showed gas bubbles in the agar. In darkness, under the same conditions, growth was slower. After nine days, starch and agar slants showed good growth; dextrose, lactose, and starch agar shakes, moderate growth, mostly at or near the surface, with no gas bubbles in the medium; growth was slight, or doubtful, in nutrient and Knop's agar shakes.

156. On the vacuome, food vacuoles, and neutral-red reaction in Vorticella, and the question of a 'complete digestive tract' in ciliates. R. P. Hall and F. W. Dunihue, New York University.

The food vacuole is formed as an expansion of the lower part of the gullet, which is later separated as a vacuole. There is no evidence for the persistence of a canal joining vacuole and gullet after separation, such as postulated by Koehring ('30). Nor is subsequent migration of food vacuoles orderly enough to demand in explanation the existence of a 'continuous digestive system' (Koehring) with successive vacuoles joined by canals.

No neutral-red globules (vacuome) were observed adherent to the developing food vacuole, nor do older vacuoles show adherent globules, as a rule. Likewise, penetration of the food vacuoles by neutral-red globules was not observed.

The neutral-red reaction is gradual. Vacuome and food vacuoles are stained first in the lower region of the cell; later on, in the rest of the organism. Koehring explains this gradual staining of food vacuoles by assuming that neutral red enters through a 'continuous digestive system.' In dividing forms, showing cessation of feeding activities, both food vacuoles and vacuome were nevertheless stained with neutral red. Hence, formation of food vacuoles is not essential to the neutral-red reaction. Observations on the formation of food vacuoles and the neutral-red reaction thus offer no evidence to support Koehring's concept of a 'continuous digestive system' in ciliates.

157. Micronuclear variation in Paramecium bursaria. Lorande Loss Woodruff, Yale University.

Studies on a race of Paramecium bursaria, in pedigree culture for more than six years, lead to the following chief results: Marked variations occurred in the micronuclear number. Originally bimicronucleate, the race later assumed the unimicronucleate condition characteristic of the species, and finally became amicronucleate. No marked variation in the vitality of the race has been observed throughout its life in culture, so that whatever function the micronuclear apparatus plays in the somatic life of the race is not obviously influenced by profound changes in the volume and distribution of the micronuclear material. Evidence of endomixis or conjugation has never been observed. The viability of amicronucleate animals, without the power to undergo endomixis or conjugation, further supports the identification of the macronucleus and micronucleus as a segregation of somatic and generative elements into discrete bodies within the cell.

MISCELLANEOUS

Read

158. The differential rate of discovery of species of genera, small and large. W. H. Longley, Goucher College. (Lantern; 15 min.)

Although the actual ranges of the vast majority of species are not known, some of the general laws of animal and plant distribution have been discovered. It is unnecessary to cite any. It suffices to say that peculiarities in distribution which obey law will make themselves manifest in the relative frequency with which species in different categories come to the hands of collectors.

This paper reports the results of a study of the differential rate of discovery of species of Chiroptera grouped by the size of their genera.

The available data is upon the whole probably as nearly adequate as that for any comparable group of organisms. Upon analysis, it shows clearly that species of large genera come to light prevailingly early; those of small genera prevailingly late; but species of monotypic genera are discovered distinctly earlier than species of genera of the next few sizes larger.

Dr. J. C. Willis and others have shown that in all large natural groups of organisms the curve of genera plotted by size is practically uniform. The data

regarding differential rate of discovery of species in correlation with generic size show that these curves from taxonomy will with the increase of knowledge approach the same limiting form.

The limit is a function of the normal curve already defined before the zoölogists. It expresses graphically the distribution by size which genera in every large natural group tend to acquire in the course of evolution.

159. The trunk muscles of snakes and their bearing on taxonomy, and phylogeny. Walter Mosauer, University of Michigan. (Introduced by the Secretary of the Society.) (Lantern; 15 min.)

The trunk muscles of snakes show considerable differences in arrangement between the three largest groups, the Boidae, Colubridae, and Viperidae, but within each family their arrangement remains stable.

The Boidae are characterized by primitive epaxial, hypaxial, and intercostal muscles and by a small muscle restricted to this group.

The colubride musculature is specialized most highly. The epaxial muscles show a tendency to abandon attachment to bones, their number is increased, and the intercostal muscles are differentiated.

The Viperidae are more primitive in their epaxial musculature; a hypaxial muscle is extremely strong, and the intercostals are arranged as in the Colubridae.

These myological main types, founded on several correlated features, are not influenced by environmental factors. Colubridae of terrestrial, arboreal, subterranean, or aquatic habits show the muscle arrangement typical of the family. This renders these features valuable for taxonomic or phylogenetic considerations.

The following conclusions are based solely on the anatomy of the trunk muscles. The primitive position of the Boidae can be confirmed. Xenopeltis is closely related to this family. Ilysia is closer to the Colubridae.

The union of aglypha, opisthoglypha, and most proteroglypha in the family Colubridae should be emphasized.

Some proteroglypha are distinct, showing the typical viperide arrangement (Naja, Sepedon, Acanthophis).

Viperidae and Colubridae may come independently from a common rather primitive ancestor; the Viperidae, through forms like Acanthophis.

The taxonomic position of Heterodon is uncertain. Typhlops deserves a quite separate position among the snakes, if it belongs to this group at all.

160. Growth and gill development in the small-mouthed bass, Micropterus dolomieu, Lacépède. John W. Price, Ohio State University. (Introduced by Raymond C. Osburn.) (Lantern; 10 min.)

Measurements of the gill surfaces of 127 specimens, ranging in total length from 1 to 27 cm., indicate that a highly correlated, geometrical relationship exists between these surfaces and external body measurements throughout the life history. After hatching, the filaments, at first few in number, multiply very rapidly until they reach a maximum, after which no more are added. The filaments grow in length, width, and depth at a constant rate throughout the life history and in direct proportion to the total length of the fish. The area of the

folded surfaces of the filaments, as well as of the smooth surfaces, varies as the square of the length of the fish. The area of these folded surfaces is expressed by the interrelation of the height of these folds; their number, per running millimeter and the length of the filament. These lamellae, at first small, increase proportionally in height, but decrease in their numbers per unit length of the filament. These various factors are so related that the gill surfaces maintain a constant relationship to the total length of the fish at all sizes, the gill surfaces increasing at a rate in excess of, but approaching, the square of the total length of the fish.

The square root of the total gill surface of the bass is equal to the cube root of the weight of the fish, multiplied by a constant. The gill surface per 1 gram of live weight is proportionally greater in younger than in older fishes.

161. Secondary sex characters in certain snakes. Frank N. Blanchard, University of Michigan. (Lantern; 10 min.)

Keel-like ridges occur on the dorsal scales of the anal region in various species of smooth-scaled snakes, such as species of Diadophis and Carphophis. In some snakes with keeled dorsal scales, the keels on the scales in the anal region bear knobs. These keel-like ridges and knobbed anal keels are practically confined to males above a certain length, varying with the species, and are interpreted as secondary sex characters. The males of some keeled species, typified by Natrix rhombifera, have also numerous tubercles in the chin region. All of these structures are assumed to be used as rubbing or stimulating organs during mating. They are useful aids in determining maturity in species possessing them and in estimating relationships between species and genera.

By demonstration

162. Specimens of Urnatella gracilis from the Licking River, Kentucky. S. R. Williams, Miami University.

Like many other zoölogists, I had read of the endoproctous bryzoan, Urnatella, and had seen the diagram which appears in the text-books. I had, however, never seen living specimens. Through the kindness of Dr. G. B. Twitchell, of Cincinnati, who personally guided the expedition, a number of us were shown this little bryozoan growing in quantity, on the under surface of rocks in the bed of the Licking River. A stream may not be polluted nor can it have too much abrasive material in its waters if this delicate creature is to thrive. A number of rocks were brought in with the hope that perhaps some of the numerous stems on these rocks might regenerate polyps. The specimens shown are contracted, since there was no opportunity for narcotization.

By title

163. The prenatal growth in weight and length of the digestive tube of the cat. Homer B. Latimer, University of Kansas.

The weight of the entire tube of 229 embryos and 35 newborn cats varies from 0.0015 to 5.64 grams. The weights in grams of the digestive tube, esophagus, small and large intestines, all form curves concave superiorly when plotted

against body weight. The weights in grams of the parts range as follows: esophagus, 0.0003 to 0.238; stomach, 0.0008 to 1.15; small intestine, 0.0002 to 3.37, and the large intestine, 0.0002 to 1.44.

The curves of the lengths of the entire tube, esophagus, small and large intestines rise rapidly during the first few grams' increase in body weight and then more slowly thereafter. The lengths in millimeters range as follows: entire tube, 16 to 635; esophagus, 4.5 to 56; small intestine, 4 to 475; large intestine, 2 to 80.

The weight of the digestive tube increases from a minimum of 0.5 per cent of the body weight to a maximum of 6.1 per cent. This increase is most rapid in the smaller embryos, or under 10 grams of body weight.

The length of the entire tube forms but 0.53 of the body length in the smallest embryo. The body length was measured along the convex dorsal side of the body from the nose to the anus. This ratio of the length of the digestive tube to the body length increases rapidly at first and then much more slowly to a maximum of 3.94 times the body length.

164. The skeletal anatomy of Pliotrema, a six-gilled pristiophorid shark. L. Hussakof, American Museum of Natural History.

Pliotrema is a rare sawshark from South Africa, similar to Pristiophorus in external appearance, except that it has six gill openings instead of five. This study of the skeleton was made to ascertain if the six-gilled condition is correlated with any archaic skeletal features. The skeleton studied is in the British Museum, and careful drawings of it were made.

There are six complete branchial arches; the sixth gill opening is a true gill. The branchial structures correspond to those of Pristiophorus, except that the branchial region is relatively more elongate, and there are differences in shape of some elements, especially the hyobranchials. The skeletal structures of the fins differ only in details from those of Pristiophorus.

Pliotrema is thus an aberrant pristiophorid, with six gills, and not a member of the archaic six- and seven-gilled family Hexanchidae.

165. The structure and function of the facial pit in the pit vipers. W. Gardner Lynn, Johns Hopkins University. (Introduced by the Secretary of the Society.)

The function of the richly innervated facial pit of the pit vipers has long been a subject of conjecture among herpetologists, but little has been done to ascertain the precise anatomical relations of the organ. A comparative study of a number of pit vipers, including representatives of all the known genera, has revealed the fact that in most forms there lies immediately beneath the pit a large cavity lined with a much-folded epithelium which is characterized by the presence of a large number of tubercles on its surface. This cavity is in communication with the air by means of a small opening at the anterior corner of the orbit. In the rattlesnake the cavity is not directly under the pit, but somewhat behind it, so that the wall of the pit is in direct contact with the maxillary bone. The ophthalmic and superior maxillary branches of the trigeminal furnish the nerve supply to this organ.

Sections of late embryos of the copperhead show that the outer lining of the pit is a somewhat modified epidermis in which are found numerous sensory cells whose connections with the ends of nerve fibers can be clearly seen.

The anatomical relations of the pit and the character of the sensory cells, as well as the results of some experimental work now in progress, seem to indicate that this organ may be the seat of a chemical sense allied to olfaction and gustation.

166. The heart of the lungless salamander. A. Grace Mekeel, Cornell University. (Introduced by Secretary of Section F.)

The heart of the adult lungless salamander is bilocular (Hopkins, '96), and what appears to be a vestigial septum atriorum is the sinu-auricular valve with altered attachments (Bruner, '00; Cords, '23).

A comparative study of the developing heart in Amblystoma and the lungless salamanders, Desmognathus and Plethodon, shows that in the former the development of the lungs precedes that of the pulmonary chamber of the heart and that in the lungless salamander a complete absence of a double auricle is correlated with the vestigial state of pulmonary development.

167. Hatching twenty-two-year-old eggs and the reduction of vigor in the rotifer Hydatina senta. David D. Whitney, University of Nebraska.

Fertilized eggs of this rotifer were produced in the fall months of 1908 in general laboratory cultures at Middletown, Connecticut. The culture jars were stored away until 1916, and then some of the culture water containing the eggs was carried to Lincoln, Nebraska. Here they have remained in storage in the original culture water until the present time. In 1916, 1928, 1929, and 1930, eggs have been removed from the old culture water and placed in rain water. Within a few days many eggs hatched. In the spring of 1930, the average number of daughters produced by fifty-two mothers from these old eggs was 27+ per mother, whereas forty-four mothers collected from a pool near Lincoln produced 57+ daughters per mother. The average length of life of the mothers from the old eggs was 4+ days, whereas the average length of life of the pond mothers was 5+ days. These two sets of observations seem to indicate a reduction in the general vigor of the strains that have remained dormant twenty-two years.

168. The retention of dye in intra-vitam methylene-blue preparations. Elbert C. Cole, Williams College.

An ideal methylene-blue dye for intra-vitam staining of nervous elements should have the following characteristics: *a*) low toxicity; *b*) a fairly high degree of solubility in physiological saline or Ringer's; *c*) very low solubility in alcohol. Methylene blue, U.S.P. Medicinal (certified), has such a low level of toxicity that satisfactory intra-vitam pictures of nervous elements are readily obtained. Its relatively high solubility in alcohol, however, means that much of the stain is extracted during the process of dehydration, even after tissues have been carefully fixed in ammonium molybdate. Methylene blue ($ZnCl_2$ double salt), although only slightly soluble in alcohol, is so highly toxic that satisfactory intra-vitam preparations can seldom be obtained.

The following procedure has been found to give unusually good results. Tissues were stained in solutions of methylene blue, U.S.P. Medicinal (certification no. NA48), and fixed in: zinc chloride, 5 grams; ammonium molybdate, 5 grams; water, 100 cc. Maceration was thereby eliminated and the loss of stain during dehydration distinctly reduced. Final preparations were practically free from distortion and retained to a large degree their original depth of stain. Although these results do not prove that the double salt of zinc and methylene blue is built up during fixation, nevertheless the sharp decrease in the solubility of the dye within the tissues is certainly suggestive.

ECOLOGY

Read

169. Ecology of certain Oriental land fishes and crustaceans. A. S. Pearse, Duke University. (Lantern; 15 min.)

Judged by the freezing-point of blood, Japanese littoral crabs present a graded series: those on land having less salt than those in the ocean. Land crabs, like vertebrates in general, apparently have less saline blood than marine animals. Siamese air-breathing fishes live in shallow water which often contains little oxygen. They have perhaps been forced to develop ability to breathe air by lack of oxygen as well as by periodic aridity. In Siam and India the land-dwelling fishes which live together on beaches avoid interspecific competition by eating different types of foods. The different species of littoral gobies have quite different types of parasites. Land vegetation has apparently by furnishing food induced crabs to migrate from the ocean to land. Land and fresh-water crabs apparently have more parasites than those in the ocean.

170. A study of fish from a small pond in a sphagnum peat bog in Ohio. R. V. Bangham, College of Wooster. (5 min.)

Fish were taken at intervals from a small pond on Cranberry Island in Buckeye Lake. These were young of yellow catfish, Ameiurus natalis; black bullhead, Ameiurus melas; carp, Cyprinis carpio; mud minnow, Umbra limi. The fish were taken at intervals until the last time in September when all fish had disappeared. Records were taken of the temperature, the pH of the water, dissolved oxygen, and carbon dioxide. These data show a decided change in these values during the summer. The rise in the degree of acidity and lowering of dissolved oxygen content account for the disappearance of the fish.

These fish all had a certain species of small parasitic nematode not found in any fish examined from the lake. Data are given for the vegetation of the pond.

171. Tagging of fish in Ohio. R. V. Bangham, College of Wooster. (10 min).

These data are obtained from fish tagged with small aluminum disks bearing a serial number and fastened in the operculum of the fish. This work was done for the Division of Conservation of Ohio, and most of the fish tagged and released were from Buckeye Lake, Ohio.

The fish were caught with a trammel net which was lifted twice daily. This net was set for ten days at intervals of one month during July, August, and September, 1930. Forty-eight hundred fish were tagged and data are given on species, length, return to net, etc. Line fishermen are given a reward for the return of tag with data on length, place caught, and some scales from the fish. The species of fish taken varied greatly in the different months and locations. There was also variation in fish coming into the net during the day and night. These data will be discussed in relation to results obtained by the operation of a gill net and seine hauls made at two-week intervals at forty stations about the lake.

172. The bottom shore fauna of western Lake Erie; a population study to a depth of 6 feet. Frederick H. Krecker and L. Y. Lancaster, Ohio University and Kentucky State Teachers College. (Lantern; 12 min.)

The densest population was in less than 36 inches of water, and 18 inches appeared to be the optimum depth. The largest total population occurred on shelving rock, due to the great numbers of chironomid larvae and Lymnaea present. Gradient appears to be an important factor in governing total population; the heaviest population occurred where the slope was most gradual. Of the vegetation considered, Scirpus had the densest population, due again to the great numbers of chironomid larvae. The number of forms was fairly uniform at all depths, with the exception of the 36-inch depth, which had the fewest forms. Sand bottom had the smallest variety of forms, whereas rubble and stone bottoms had the largest variety. Among the plants, Scirpus had the fewest forms. There was no general correlation between gradient and number of forms. The results obtained are based on studies carried on at the Franz Theodore Stone Laboratory during the past several summers, and especially during the last two summers. The area covered was the western end of Lake Erie, and typical habitats selected were the following: Exposed smooth shelving rock, cliff-boulder shore, exposed flat-stone shore, shelving rock rubble deposits, exposed rubble shore, exposed rubble bar, protected rubble bar, rock pools, gravel shore, exposed bare sand beach, hard clay Scirpus-Potomageton shore, Scirpus clay shore, Scirpus-Potomageton sand beach, Potomageton sand shoal.

173. Notes on food and winter habits of Triturus viridescens. Ann H. Morgan and Margaret Grierson, Mount Holyoke College. (Lantern; 8 min.)

These conclusions regarding the food of Triturus viridescens were based upon the study of over 500 adults collected from two slow streams of the same region, twelve to twenty or more animals being taken in each month, except September.

Animals captured in March contained the least food; those taken in August contained the most. In March, a total of twenty salamanders contained four insects; in August, twelve salamanders contained 179 insects and forty-eight crustaceans. In October, the proportion of insects fell and the number of crustaceans rose. The various organisms found were identified and counted. In general, the diet of the Triturus consisted of insects, cast skins (Triturus), crustaceans, mollusks.

Observations were made upon the winter habits of Triturus in the same streams.

174. Territory in mammal life. W. L. Strunk, University of Michigan. (Introduced by the Secretary of the Society.) (15 min.)

The researches of such men as Howard, Friedmann, and others on birds have shown that a territory as such is to be found in that group of animals. Other investigators have shown that a similar condition prevails in fishes, reptiles, amphibians, etc. The present work was begun in an attempt to determine the presence or absence of such a condition in mammals. By far the greatest amount of work was done on Microtus pennsylvanicus, the common meadow mouse. The system followed in the study was briefly this: The animals were caught in special traps, which permitted them to be taken alive. The individuals on being caught were marked in such a way that they could be re-identified on being recaught. The marking method was based on a system of toe amputation and ear punching. After marking and taking of the proper data, such as date, age, sex, abnormalities, if any, etc., the individuals were released either at or away from the trap. The traps were set in series, in proper locations, and each was numbered and its position plotted on a map. By use of this method the movements of some 860 animals were followed and the extent of their territorial ranges determined.

By demonstration

175. The fauna of Grand Isle, Louisiana: Preliminary survey of the free-living fauna exclusive of protozoa and mammals. Ellinor H. Behre, Louisiana State University.

Most of the larger groups are well represented. The fauna, though very varied, is not very rich; there are almost as many genera within each group as there are species.

Interesting features are: 1) The very great fluctuation in abundance of certain forms, notably the Coelenterata. The fluctuations are not obviously correlated with other changes in the immediate environment, such as certain winds or weather or changes in salinity or turbidity of the water. 2) The great scarcity of worms and echinoderms. 3) The lack of anything unusual in insect forms. 4) The tremendous seasonal swarming of Ctenophora, especially Mnemiopsis, probably though not positively associated with breeding habits. 5) The very wide spread of many of the embayment fishes over open gulf regions, due probably to the shallowness of the off-shore waters and consequent overlap of the habitats of closely related groups. 6) Further confirmation of earlier observations to the effect that this locality, though very rich in birds of passage at the migrating seasons, is at other times sparsely populated, in spite of a well-developed ridge of dense trees running the length of the island through its center. This applies also to water birds. There are only small feeding grounds at both ends of the island and almost no nesting, possibly due to the nearness of human habitations.

176. The frogs of the United States and Canada. Albert Hazen Wright and Anna Allen Wright, Cornell University.

This is a part of a plan of treatment for the frogs, turtles, lizards, and snakes of the United States and Canada. Herewith appear the plates of the frogs. They are photographs from life.

In all there are 108 plates of 1143 photos. The attempt is to present salient facts both pictorially and textually. The general treatise consists of 32 plates, 347 photos illustrating the following topics: size, parts, lateral aspects, secondary sexual characters, enlarged tympana, throat colorations, croaking, types of egg masses, egg masses in particular, tadpoles, tadpole mouth parts, development, transformation, hibernation. The 76 plates of 796 photos in general represent one plate for each of the seventy-six forms of the U. S. A. and Canada. Each plate may have from five to twenty photos representing various aspects of adults, habitats, secondary sexual characters, eggs, transformation. In addition, there are five colored plates of Ascaphus truei, Bufo alvarius, Bufo canorus, Hyla cinerea, and Rana aurora aurora. Sample plates of lizards, turtles, and snakes also appear.

177. Methods for ecological studies on birds. S. Prentiss Baldwin and S. Charles Kendeigh, Baldwin Bird Research Laboratory, Cleveland. (Introduced by L. J. Cole.)

For some years this laboratory has been working in physiological and life-history studies of living wild birds, making special use of the house wren. About 400 wren boxes are kept under observation on sixty-five estates over some 3000 acres, and some 1200 wrens are handled in a season.

Devices for mechanical recording have greatly added to the number and accuracy of observations. Multiple and portable 'itographs' have been devised which register every visit of adult birds to the nest, by means of perches making electric contacts. These run continuously and record amount of incubation, care of young, and other nest activities.

The physiological responses of birds to the environment are being studied. Thread thermocouples placed over eggs in nest obtain temperature applied for incubation and skin and body temperature of incubating adult. These temperatures are recorded by potentiometers that run automatically day and night. Correlation of fluctuations in body and nest temperatures with air temperatures and humidities is obtained by use of hygrothermographs placed near the nest.

In cooperation with Doctor Sawyer, president of The Brush Laboratories Company, an apparatus is being worked out for studying body pulsations and vibrations due to internal movements of small animals. Such movement may originate with the heart, the respiratory organs, or intestinal tract. Sensitive parts of such apparatus in contact with the animal must not cause fright or interfere with free movement. For birds, a piezo-crystal perch combines sufficient sensitivity and freedom from background noises, such as microphone hiss, to permit direct connection with the galvanometer of a cardiograph recorder or an amplifier leading to loud-speaker or other apparatus.

The territorial relations of individual birds of the same species with the same ecological community are studied by means of special maps.

These maps and instruments above mentioned may be exhibited and shown in the way they are used.

By title

178. The life cycle of the California oyster (Ostrea lurida). W. R. Coe, Yale University.

With the cooperation of Prof. W. E. Allen, of the Scripps Institution of Oceanography, the season of attachment and rate of growth of various sedentary organisms, including the oyster, have been determined during four successive years. By means of wood and cement blocks placed in the water at stated intervals throughout the year and examined at periods ranging from one to forty weeks, reliable information has been obtained in regard to the increment of growth at different seasons and the sequence of development of the sexual products.

The spawning period of the oyster at La Jolla extends from April to October, with a season of maximum production in June and July. Each individual is hermaphroditic and protandric, the fertilized eggs developing within the mantle cavity until after the veligers have formed bivalve shells. Several successive crops of young are produced by each parent during the spawning season. Growth of the young attached mollusks is very rapid during the warmer months of the year, individuals in a favorable environment sometimes reaching a length of 25 mm. within three months. Sexual maturity may occur at an age of less than six months, when the shell has reached a length of 30 to 40 mm. It is thus quite possible that two generations may be completed within a year. As a rule, however, the young of one summer do not spawn until the following season, for only the first broods in the spring reproduce during the same calendar year. Overlapping generations and prolonged spawning periods result in the continuous production of larvae during the seven warmer months of the year.

PARASITOLOGY

Read

179. Notes on the life history of a proteocephalid from Rana clamitans. Lyell J. Thomas, University of Illinois. (Lantern; 10 min.)

Proteocephalids are rare in frogs, and this new species of tapeworm taken during the summer of 1930 at Douglas Lake, Michigan, is being described here. The eggs of this tape when laid have fully developed hexacanth embryos within gelatinous embryophores. Cyclops leuckarti, C. viridis, and C. albidus ingest them. Mature procercoids were found only in C. viridis, var. brevispinosus. In the body cavity of the copepod the scolex develops five fully formed suckers which are invaginated within the procercoid. The apical fifth sucker becomes vestigial in the adult tape, but is still equal in diameter to the other suckers in the youngest tapes taken from frogs. The six hooklets are not constricted off with the cercomer. Fourteen days were required for completely developed procercoids. Direct development from infested copepods eaten by frogs seems possible.

180. The excretory system as a method of classification of digenetic trematodes. Ernest Carroll Faust, Tulane University Medical School. (Lantern; 15 min.)

The present system of classification of Platyhelminthes, including both free-living and parasitic groups, is based on external features and on the reproductive

organs. In certain groups convergent evolution has resulted in the development of similar reproductive organs in species which belong to fundamentally different families and even superfamilies. This is particularly true in the Digenea, where complicated life cycles have provided many opportunities for both divergence and convergence.

Although Looss contributed much to our knowledge of the excretory system, analyzing with particular care the solenocyte pattern, he apparently did not conceive of its fundamental importance in classification. Sinitsin ('11) and Dollfus ('30) have placed particular emphasis on the character of the primary collecting tubules as a criterion for demonstrating relationships, while Cort, Sewell, LaRue, McCoy, and Faust have demonstrated the usefulness of the solenocyte pattern in developing a natural classification of both fundamental and subsidiary groups. Thus the Gasterostomata have a fundamental pattern which may be expressed by the formula: $2[1+1]$; the Aspidogastrata, by the formula: $2[1+1+1]$; the Strigeata, by the formula: $2[1+1]$; the Monostomata, by the formula: $2[1]$; the Amphistomata, by the formula: $2[1]$; and the Distomata, by the formula: $2[1]$. Examples from superfamilies, families, genera, and species of these several groups are cited to demonstrate the value of this schema.

181. A comparative study of the viability of the ova of Diphyllobothrium latum from different hosts. H. E. Essex and T. B. Magath, Mayo Clinic. (Lantern; 10 min.)

A study of the ova of D. latum has been conducted under a variety of conditions. It has been found that, under fairly uniform conditions, more than 80 per cent of the ova from human cases develop, while about 90 per cent of the ova from dogs fail to produce coracidia.

182. Some studies on the change in virulence of a strain of Trypanosoma equiperdum, using albino rats as hosts. T. C. Carter, Northwestern State Teachers College and University of Wisconsin. (Introduced by N. R. Stoll.) (15 min.)

In order to determine any changes in virulence during the different stages of infection in a single host, it seemed necessary to determine, experimentally, the effect of varying sizes of inoculating doses on the survival time of infected rats. Three series of inoculating doses were used containing 500, 5000, and 50,000 parasites, respectively. Some forty rats were used. Results showed that the smaller the size of the inoculating dose, the longer was the survival time of the host. A standard dose of 5000 trypanosomes was accordingly used in the subsequent studies.

Further studies showed that the virulence of the parasites decreased as the stages of infection advanced in each host. Subinoculations were made from the same infected rat at twenty-four-hour intervals beginning at the first appearance of trypanosomes in the peripheral blood and continuing until the death of the host. The average survival time of rats infected with parasites at their first appearance in the donor was 133 hours. Those infected with trypanosomes taken after their appearance in the blood of the donor twenty-four hours,

survived 157 hours; while those animals subinoculated with trypanosomes from rats at death (i.e., forty-eight hours after the first appearance of the parasites in the blood) survived 175 hours. These studies show that the virulence was reduced approximately one-third during the residence of the parasites within the donor host.

183. The validity of Taenia confusa Ward, a tapeworm of man. Marlowe G. Anderson, Northwestern University. (Introduced by F. D. Barker.) (Lantern; 7 min.)

In a comparative study of the general morphological and histological characteristics of forty-one specimens of Taenia saginata it is shown that in every detail described as being specifically different in the tapeworms of man, Taenia confusa and Taenia saginata, there is an intergradation of variations between the two forms. According to the accepted definition of a species, there can be no such chain of variations between forms which are distinct species.

New limits in various measurements and other characteristics of Taenia saginata are given and attention called to the error of creating new species on the basis of a limited number of specimens.

184. The biochemistry of the sheep tapeworm Moniezia expansa. Otto A. Tischer, Northwestern University. (Introduced by F. D. Barker.) (Lantern; 8 min.)

The investigations as previously reported ('29) were continued, and the results show that the tissue of the tapeworm Moniezia expansa is made up of the following organic constituents: Carbohydrates, 39.616 per cent; N-containing substances (protein), 36.506 per cent; fats, 19.39 per cent, and purine bases, 00.345 per cent. All calculations were based upon a total dry material of 10 per cent. It is of greater interest to note that glycogen represents the largest portion of the chemical constituents, while proteins and fats follow in the order mentioned. From the protein molecule the following amino-acids could be isolated: glycine, 4.914 per cent; alanine, 7.742 per cent; valine, 0.308 per cent; glutamic acid, 2.736 per cent; arginine, 0.109 per cent; lysine, 0.068 per cent; phenylalanine, 5.928 per cent; tyrosine, 6.692 per cent; histidine, 0.401 per cent; proline, 1.030 per cent; tryptophane, 1.408 per cent, and peptide anhydrites, 0.051 per cent. The analysis of the fat fraction revealed the presence of total lipins, 18.195 per cent (lecithin, kephalin, and galactolipins); unsaturated fatty acids, 49.518 per cent (lower fatty acids and oleic acid); saturated fatty acids, 20.617 per cent (stearic acid and palmitic acid); sterols, 8.249 per cent (cholesterol), and glycerine, 3.271 per cent. Purine bases were found in the form of creatine, 0.119 per cent; adenine, 0.076 per cent; hypoxanthine, 0.103 per cent, and a base not reported before. An elementary analysis of this base, which was isolated in the form of the platinum salt, gave: C, 19.69 per cent; H, 3.29 per cent; N, 5.12 per cent; Pt, 31.49 per cent, which suggests an empirical formula of $(C_6H_{10}NOCl)_2PtCl_4$ with a molecular weight of 619.97 and a melting-point of 229–230°C.

This biochemical investigation gives additional information on the parasitic habits of the tapeworm and its effects upon the host when considering especially the presence of the large amounts of essential amino-acids of great nutritional value.

Experiments with cultures and the results obtained are reported.

185. The effect of certain environmental factors on the development and hatching of the eggs of blood flukes. Angelo R. Oronato and Horace W. Stunkard, New York University. (7 min.)

The miracidia are positively phototrophic. The contents of the egg, which flow out of the shell with the larva, form a membrane which prevents the miracidium from escaping at once into the water. The protein in this substance is probably the cause of the membrane formation. Ordinary tap-water is most suitable for the development and hatching of these eggs. Putrid water is toxic to the larvae. If the water in the culture is allowed to evaporate, the larvae die in a few minutes. The most favorable pH zone for hatching of these eggs lies between 7.2 and 7.6. The miracidia cannot live in a solution having a pH lower than 6.8 or higher than 8. The optimum temperature for development and hatching is the range 20° to 25°C. Exposure to temperature of 0°C. for five hours had a fatal effect upon the miracidia. Although they became quiescent at a temperature of 10°C., they recovered from it on return to room temperature. After extended exposure to temperatures higher than 40°C., the larvae do not recover.

186. The effect of dilution of sea-water on the activity and longevity of the cercariae of Cryptocotyle lingua. Horace W. Stunkard, New York University. (8 min.)

An attempt is made to secure evidence bearing on the evolutionary history of trematode species belonging to a common family, some of which parasitize marine, while others infest fresh-water hosts. The family Heterophyidae contains several species whose larval stages infest fresh-water snails and others, e.g., Cryptocotyle lingua, in which the asexual multiplication occurs in marine snails. The larvae leave the snails to seek the second intermediate host, and consequently their activity as well as longevity was recorded. Ordinarily the cercariae live about twenty-four hours in sea-water, and some of them swim for twenty hours. In one-half sea-water they remain alive about as long as in undiluted sea-water, although their activity is somewhat reduced after twelve to fifteen hours. In one-fourth sea-water a few cercariae were swimming at the end of six hours, although most of them were dead. In one-eighth sea-water a few were swimming at the end of three hours, and about one-half were dead at the end of four hours. Freshly emerged cercariae manifest normal swimming movements for only a few minutes when placed in tap-water, the bodies and tails soon become swollen, and they live only about an hour. Adolph ('25) has shown that marine organisms have a much greater tolerance for diluted sea-water than fresh-water species have for solutions containing sea-water. If similar results obtain for cercariae, it would constitute presumptive evidence that members of the Heterophyidae, at least, were primitively marine.

187. An addition to the list of acanthocephalan parasites of Amphibia. Benjamin P. Young, Cornell University. (Lantern; 7 min.)

A description of a new species of acanthocephalan found in Necturus maculosus (the mud-puppy) with some notes as to frequency of appearance and location in the host's tissues.

188. Collyriclum faba as a parasite of poultry. William A. Riley, University of Minnesota.

Although known to infest a wide variety of passerine birds, both in Europe and in this country, there are only three records of the occurrence of the trematode Collyriclum faba in poultry. Riley and Kernkamp reported its presence in a large number of ducks on a farm on the shores of Lake Minnetonka, near Minneapolis, Minnesota, and in turkey poults in Ottertail County, Minnesota, more than 160 miles distant from the first focus. Diligent search failed to reveal other cases on neighboring farms, and the breaking up of the flocks in both instances prevented further observations. In 1926, Marotel reported the infestation epizootic among turkey poults at Romans, province of Drome, in southeastern France. Five new localities are now reported, and additional evidence supports the view that the life cycle is not atypic and that the second intermediate host is an odonate nymph.

By demonstration

189. Another trematode with two anal openings. Horace W. Stunkard, New York University.

La Rue 1926 found that in Hemistomum haustrum MacCallum, 1918, the intestinal ceca open to the surface of the body, and he erected the genus Diploproctodeum to contain it. Odhner 1928 found the same condition in Schistorchis carneus Lühe 1906 (Pleorchis oligorchis n.sp., S. J. Johnston, 1913). A reexamination of the trematode described by Linton 1898 as Distomum sp. from Lagocephalus laevigatus at Woods Hole, Massachusetts, and reported later, Linton 1905, from Spheroides maculatus, Siphostoma fuscum, and Cynoscion regalis at Beaufort, North Carolina, shows that in this species also the intestinal ceca communicate with the exterior by anal openings. For the species the name Bianium concavum is proposed.

By title

190. Vitamin requirements of intestinal nematodes. James E. Ackert, Kansas State Agricultural College and Molteno Institute, University of Cambridge, England.

The assumption of early workers that helminths require the same food accessories as their hosts is not supported by the results of recent investigations by the writer and his associates in testing vitamins A, B (complex), and D as factors in the resistance of chickens to the intestinal nematode, Ascaridia lineata (Schneider). Chickens, given diets adequate, except for the vitamin in question, and parasitized with given numbers of embryonated eggs of A. lineata, retained as many larvae after three weeks as did those chickens (brood mates) whose diets were the same except for the addition of the vitamin in question. Although most of the chickens on the deficient diets developed the typical deficiency diseases (ophthalmia, vitamin A; polyneuritis, vitamin B; and leg weakness, or rickets, vitamin D), the young A. lineata were able to grow as rapidly in these hosts as in those to which the respective vitamins were supplied. These results indicate that A. lineata does not require any of these vitamins during the first third of its growth period.

191. Two new larval nematodes from Rana sp. A. C. Walton, Knox College.

The description of two new larval ascarids and their comparison with other known larval forms found in similar hosts are presented with the hope that their description may lead to the eventual identification of the adult forms which probably occur in some of the water birds.

192. An amphistome cercaria, Cercaria poconensis, with branched main excretory tubes. Charles H. Willey, New York University. (Introduced by the Secretary of the Society.)

Sixteen snails of the species Helisoma antrosa were infected with a new amphistome cercaria, C. poconensis, belonging to the Diplocotylea group. The excretory system shows branching of the main excretory tubes in a manner hitherto unknown among the amphistomes. The excretory system is considered one of the most conservative systems in the trematode body plan, and is therefore valuable in establishing natural relationships. Each main excretory tube in the region between excretory vesicle and the level of bifurcation of the gut receives three lateral diverticula which extend laterad and forward some distance and two similar medial diverticula. After receiving several additional branches from around the eye spots, the main tubes pass medial to these structures and, at the level of the 'pharyngeal pouches,' loop back to receive anterior and posterior secondary collecting tubules. The branches as well as the main tubes are filled with many typical, large, globular concretions which in the living cercaria can be seen to pass back and forth from one tube to another. Due probably to the presence of much black pigment, no distribution from the distal ends of the branches could be identified. Development occurs in small rediae having no locomotor appendages. The excretory system of the redia shows six pairs of large flame cells. The cercariae emerge from the redia in an immature state, complete their development within the mollusc host, and emerge on bright days only. After several hours, they encyst on vegetation or on the light side of the tank.

193. The occurrence of Typhlocoelum flavum in North America. Charles H. Willey, New York University. (Introduced by the Secretary of the Society.)

In response to my request for the loan of several specimens of Zygocotyle lunata, the Bureau of Animal Industry at Washington, D. C., kindly sent three worms. They were labeled Zygocotyle lunata—Catalogue no. 27956—from Marila americana taken at Bush River, Maryland, by Chapin in January, 1924. Upon examination, the specimens proved to be monostomes rather than amphistomes. Sections showed intestinal crura anastomosing posteriorly and possessing medial diverticula, and revealed the presence of a rudimentary ventral sucker. The testes, situated at the posterior end of the worm, were distinctly lobular. The worms belong to the family Cyclocoelidae and proved to be specimens of Typhlocoelum flavum Mehlis—a species hitherto not reported from North America.

194. The life history of Epibdella melleni MacCallum 1927, a monogenetic trematode parasitic on marine fishes. T. L. Jahn and L. R. Kuhn, New York University and The New York Aquarium. (Introduced by H. W. Stunkard.)

The adult of Epibdella melleni MacCallum 1927 is found attached to the epidermis, to the conjunctiva, and sometimes in the gill and nasal cavities of marine fishes. Tetrahedral eggs, usually bearing one long and two short filaments, are laid singly and are passed into the sea-water. In six to eight days an opening appears in the non-filament-bearing corner of the egg, and a fusiform larva emerges. The larva is about 100 μ in length and has two small anterior suckers, one mouth sucker in the anterior part of the middle third of the body, and one large posterior sucker which bears six large definitive and fourteen larval hooks. The digestive tract consists of two laterally placed caeca extending posteriorly from the mouth; two large excretory vesicles are located just behind and lateral to the mouth. Two pairs of black eye spots are located anterior to the mouth.

The larva apparently becomes attached to the mucous covering of any susceptible fish and undergoes a gradual development into the adult form. The cilia are lost, the digestive tract becomes greatly diverticulated, and reproductive organs are developed. As a result of unequal growth in various regions of the body, the mouth, eye spots, and excretory vesicles assume a more anterior position. The six definitive hooks increase in size, but the larval hooks fail to increase and are very inconspicuous in the adult. Studies on the excretory system and histology of the organism are in progress.

195. Specificity of the infectious myxoma virus of rabbits. Roscoe R. Hyde and Raymond E. Gardner, School of Hygiene and Public Health, Johns Hopkins University.

Infectious myxoma is a specific disease of rabbits belonging to the group of filterable viruses first described in 1898 by Sanarelli. This disease is of special interest, since it is highly infectious, presenting both the picture of necrosis and of tumor growth. It has killed without exception more than 400 rabbits in our laboratory within from one to two weeks after inoculation. All of our common laboratory animals are refractory to the disease, although Sanarelli claims to have transferred it to a dog. We have failed to transfer the disease to the dog in repeated attempts with twelve different animals. Curiously enough, we have been unable to transfer the disease to the jack rabbit of the species Lepus californicus melanotis. In the tissues of the affected host characteristic inclusion bodies are found. Lipschütz identified the affected cells with the histiocytes. We have found, however, that the histiocyte is not affected, as can be shown by injecting myxoma rabbits with lithium carmine. In smear preparations of myxoma tissue from animals thus treated the large myxoma cells are found alongside of the unaffected carmine-filled histiocytes. Since the histiocyte is excluded, it becomes evident from extended cytological studies that the affected cell is primarily the fibroblast, although other types may be involved.

The myxoma virus has not only a high degree of specificity for the species, but also a given type of cell of the susceptible species. The fact that the fibroblast is affected to the exclusion of the histiocytes and the fact that the rabbit shows no immunity to the disease, either active or passive, may be significant.

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PROGRAM AND ABSTRACTS FOR THE JOINT GENETICS SECTIONS OF THE AMERICAN SOCIETY OF ZOÖLOGISTS AND THE BOTANICAL SOCIETY OF AMERICA

Chairman, L. J. Cole, University of Wisconsin, Madison, Wisconsin
Secretary, P. W. Whiting, University of Pittsburgh, Pittsburgh, Pennsylvania

PROGRAM OF THE TENTH ANNUAL MEETING, DECEMBER 29, 1930–JANUARY 2, 1931

Monday Morning Session, December 29th, 10.00 a.m.

Lecture Room, Biology Building, Western Reserve University

Joint session with the Geneticists Interested in Agriculture.

Symposium: Inbreeding and its application to improvement in plants and animals.

1. The value of inbreeding in asexually propagated crops, with special reference to the potato, Solanum tuberosum. F. A. Krantz, University of Minnesota, Minneapolis, Minnesota.
2. The application of inbreeding to physiological studies in animal breeding. W. F. Dove, Maine Agricultural Experiment Station, Orono, Maine.

Monday Afternoon Session, December 29th, 2.00 p.m.

Lecture Room, Biology Building, Western Reserve University

Joint session with Section O, A.A.A.S., and the American Society of Agronomy.

1. Inbreeding with particular reference to maize. R. J. Garber, West Virginia University, Morgantown, West Virginia.
2. Experiments on hybrid vigor and convergent improvement in corn. F. D. Richey and George F. Sprague, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

Tuesday Morning Session, December 30th, 10.00 a.m.

Lecture Room, Biology Building, Western Reserve University

1. Facet number and genetic growth constants in bar-eyed stocks of Drosophila. A. H. Hersh, Western Reserve University, Cleveland, Ohio. (Lantern; 10 min.)
2. The effect of long subjection to constant temperatures upon bar eye of Drosophila melanogaster. 1930 report. Charles Zeleny, University of Illinois, Urbana, Illinois. (Lantern; 10 min.)

3. The change from monoecism to dioecism in maize. Donald F. Jones, Connecticut Agricultural Experiment Station, New Haven, Connecticut. (15 min.)
4. Hybridization of Tripsacum with Zea mays and Euchlaena mexicana. P. C. Mangelsdorf and R. G. Reeves, Texas Agricultural Experiment Station, College Station, Texas. (Lantern; 10 min.)
5. The meiotic phenomena in Drosophila melanogaster. Bessie B. League, University of Texas, Austin, Texas. (Introduced by T. S. Painter.) (Lantern; 8 min.)
6. Chromosome numbers in the Cucurbitaceae. Thomas W. Whitaker, University of Virginia, University, Virginia. (Introduced by Orland E. White.) (10 min.)
7. The chromosomes of Clarkia. L. L. Burlingame, Stanford University, California. (Lantern; 15 min.)
8. On the cytology of Matthiola incana in relation to the inheritance of double flowers. J. Philp and C. L. Huskins, McGill University, Montreal. (15 min.)
9. A cytological demonstration of the location of an interchange between two non-homologous chromosomes of Zea mays. Barbara McClintck, Cornell University, Ithaca, New York. (Lantern; 15 min.)
10. The occurrence of unpaired chromosomes in hybrids between varieties of Triticum vulgare. Lillian Hollingshead, Dominion Rust Research Laboratory, Winnipeg, Manitoba. (Introduced by E. B. Babcock.) (Lantern; 10 min.)

TUESDAY AFTERNOON SESSION, DECEMBER 30TH, 2.00 P.M.

Laboratory, Biology Building, Western Reserve University

Demonstrations and exhibits

Species crosses in Aquilegia. Edgar Anderson, Missouri Botanical Garden and Washington University, St. Louis, Missouri.

Factor interactions in Primula sinensis. Edgar Anderson, Missouri Botanical Garden and Washington University, St. Louis, Missouri.

Genetics herbarium. Edgar Anderson, Missouri Botanical Garden and Washington University, St. Louis, Missouri.

Chromosome studies in the genus Sorghum. C. L. Huskins and S. G. Smith, McGill University, Montreal.

Microscopic demonstration of the chromosomes of Clarkia. L. L. Burlingame, Stanford University, California.

Microscopic demonstration of cytological evidence of genetical relationships in Oenothera. Ralph E. Cleland, Goucher College, Baltimore, Maryland.

New mutations in Habrobracon. Kathryn A. Gilmore, University of Pittsburgh, Pittsburgh, Pennsylvania. (Introduced by P. W. Whiting.)

Translocations, deletions, and breakage in Drosophila melanogaster. T. S. Painter, University of Texas, Austin, Texas.

A lethal defect in development of the chick embryo. T. C. Byerly and Morley A. Jull, Bureau of Animal Industry, U. S. Department of Agriculture.

WEDNESDAY MORNING SESSION, DECEMBER 31ST, 10.00 A.M.

Lecture Room, Biology Building, Western Reserve University

11. The frequency of mutation of specific genes in maize. L. J. Stadler, United States Department of Agriculture and University of Missouri. (Lantern; 15 min.)
12. The relation between x-ray intensity and the frequency of deficiency in the maize endosperm. S. F. Goodsell, University of Missouri, Columbia, Missouri. (Introduced by L. J. Stadler.) (Lantern; 10 min.)
13. Some genetic effects of electromagnetic treatments in maize. George F. Sprague, Bureau of Plant Industry, Washington, D. C. (10 min.)
14. Pollen-tube growth and seed production after radium treatment. J. T. Buchholz, University of Illinois, Urbana, Illinois, and A. F. Blakeslee, Carnegie Institution of Washington, Cold Spring Harbor, Long Island. (Lantern; 12 min.)
15. F_1's from radium-treated pollen. A. F. Blakeslee, S. Satina, A. D. Bergner, J. S. Potter, and A. G. Avery, Carnegie Institution of Washington, Cold Spring Harbor, Long Island. (Lantern; 15 min.)
16. The effect of ultraviolet radiation on mutation. Edgar Altenburg, The Rice Institute, Houston, Texas. (15 min.)
17. Disorganization of wing veins caused by x-raying larvae of Habrobracon. Anna R. Whiting, Pennsylvania College for Women, Pittsburgh, Pennsylvania. (Lantern; 10 min.)
18. A study of mutations, mosaics, and non-inherited abnormalities among progeny of individuals x-rayed as larvae in Habrobracon. Anna R. Whiting, Pennsylvania College for Women, Pittsburgh, Pennsylvania, and Carey H. Bostian, North Carolina State College, Raleigh, North Carolina. (Lantern; 5 min.)

WEDNESDAY AFTERNOON SESSION, DECEMBER 31ST, 2.00 P.M.

Laboratory, Biology Building, Western Reserve University

Demonstrations and exhibits.

THURSDAY MORNING SESSION, JANUARY 1ST, 10.00 A.M.

Lecture Room, Biology Building, Western Reserve University

19. Evidence of the divisibility of the gene. I. J. Agol, Timiriazef Biological Institute, Moscow, U.S.S.R., and University of Texas, Austin, Texas. (Introduced by H. J. Muller.) (15 min.)
20. Complex gene rearrangements induced by x-rays. C. P. Oliver, Washington University, St. Louis, Missouri. (Introduced by Caswell Grave). (Lantern; 15 min.)
21. An egg lethal associated with the pervirens factor in Oenothera. George H. Shull, Princeton University, Princeton, New Jersey. (Lantern; 15 min.)
22. Cytological evidence of genetical relationships in Oenothera. Ralph E. Cleland, Goucher College, Baltimore, Maryland. (15 min.)

23. The genetics and cytology of a tetraploid sport from Oenothera franciscana. B. M. Davis, University of Michigan, Ann Arbor, Michigan. (Lantern; 15 min.)
24. The relation of size to shape in the heredity and development of Cucurbita fruits. Edmund W. Sinnott, Barnard College, Columbia University, New York, New York. (Lantern; 10 min.)
25. Genetic control of susceptibility to the inoculated mouse leukemias of lines I and L. E. C. MacDowell, Carnegie Institution, Cold Spring Harbor, Long Island, and M. N. Richter, Department of Pathology, College of Physicians and Surgeons, New York, New York. (Lantern; 15 min.)

THURSDAY AFTERNOON SESSION, JANUARY 1ST, 2.00 P.M.

Laboratory, Biology Building, Western Reserve University

Demonstrations and exhibits.

FRIDAY MORNING SESSION, JANUARY 2ND, 10.00 A.M.

Lecture Room, Biology Building, Western Reserve University

26. Differential reaction in disease resistance within two highly inbred strains of rats and the reaction of their hybrid progeny. M. R. Irwin, University of Wisconsin, Madison, Wisconsin. (15 min.)
27. A critique of the various hypotheses of the inheritance of the blood groups. Laurence H. Snyder, Ohio State University, Columbus, Ohio. (Charts; 15 min.)
28. The phenotypic expression of the mutant, squatty, in Drosophila hydei. W. P. Spencer, College of Wooster, Wooster, Ohio. (Charts; 10 min.)
29. A generalized theory of four-strand crossing over. Alexander Weinstein, The Johns Hopkins University, Baltimore, Maryland. (15 min.)
30. A triple allelomorph in doves and its interspecific transfer. L. J. Cole, University of Wisconsin, Madison, Wisconsin. (Lantern; 15 min.)
31. Further studies on inheritance in Cladocera. A. M. Banta and Thelma R. Wood, Carnegie Institution, Cold Spring Harbor, Long Island, and Brown University, Providence, Rhode Island. (Lantern; 15 min.)
32. The production of high lens-antibody content in rabbits. Heman L. Ibsen and L. D. Bushnell, Kansas State Agricultural College, Manhattan, Kansas. (Lantern; 15 min.)
33. The hereditary basis for melanosis in hybrid killifishes. Myron Gordon, Zoölogical Laboratory, Cornell University, Ithaca, New York. (Introduced by H. D. Reed.) (10 min.)

Papers to be read by title

43. Genetic results from conjugation of double monsters and free individuals of Paramecium caudatum. Charles F. DeGaris, The Johns Hopkins University, Baltimore, Maryland.
44. Nucleus versus cytoplasm in the heredity of Paramecium caudatum as shown by conjugation of double monsters. Charles F. DeGaris, The Johns Hopkins University, Baltimore, Maryland.
45. Analysis of several induced gene rearrangements involving the X-chromosome of Drosophila. H. J. Muller and W. S. Stone, University of Texas, Austin, Texas.
46. Mutations and freaks from x-rayed adult males of Habrobracon. R. Elizabeth Chalmers, University of Pittsburgh, Pittsburgh, Pa. (Introduced by P. W. Whiting.)
47. The production of mosaics in Drosophila by x-rays. J. T. Patterson, University of Texas, Austin, Texas.
48. On the occurrence of so-called sex chromosomes in hermaphrodite animals. E. C. Jeffrey, Harvard University, Cambridge, Massachusetts.
49. Melanotic tumors in certain hybrids of Mexican killifishes. H. D. Reed, Zoölogical Laboratory, Cornell University, Ithaca, New York.

ABSTRACTS

Read

1. Facet number and genetic growth constants in bar-eyed stocks of Drosophila. A. H. Hersh, Western Reserve University. (Lantern; 10 min.)

Analysis of data on the dorsal (x) and ventral (y) lobes of the eye of fifteen bar stocks, differing from one another in regard to the Xple genes (scute, echinus, crossveinless, cut, vermilion, garnet), discloses a power function, $y = bx^k$, in which b and k (genetic growth constants) take different values in the different stocks; they differ also in males and females. b is a decreasing exponential function of k, giving about 36 per cent and about 43 per cent decrease per 0.1 unit increase in k in females and males, respectively. Mean facet number for the entire eye plotted against k gives a sine curve with decreasing amplitude which oscillates quite regularly (in females, at least) about the average for forked bar. For females the amplitude is about 30 facets; logarithmic decrement about 0.062; one full cycle covers about $0.20k$. Similar values for the males follow: amplitude about 50 facets; logarithmic decrement about 0.056; one full cycle covers about $0.25k$. An interpretation of the oscillating function is given in terms of the distribution of growth. The point for the beginning of facet formation in the optic disc at the beginning of the effective period shifts back and forth within a small circular arc which (projected forward, as it were) in terms of adult structure has a radius given by the amplitude; as k increases this area decreases exponentially by a factor which is the logarithmic decrement. By extrapolation, the point may be considered as fixed at $k = 1.8$.

2. The effect of long subjection to constant temperatures upon bar eye of Drosophila melanogaster. 1930 report. Charles Zeleny, University of Illinois. (Lantern; 10 min.)

Females in the 195th generation at 27° have 49.76 ± 0.56 eye facets and males, 60.46 ± 0.74 facets, while females one generation at 27° after 80 generations at 17° have 52.16 ± 0.84 facets and males, 56.70 ± 0.80 facets. Females one generation at 22° after 195 generations at 27° have 67.56 ± 0.89 facets and males, 85.10 ± 1.07 facets, while females one generation at 22° after 80 generations at 17° have 72.84 ± 0.71 facets and males, 83.20 ± 0.85 facets. Difference in temperature history seems to have produced no change in hereditary constitution other than that expressed in the degree of sexual dimorphism.

3. The change from monoecism to dioecism in maize. Donald F. Jones, Connecticut Agricultural Experiment Station. (15 min.)

A single recessive factor, silkless, sterilizes the pistillate flowers of maize. Such plants are functionally males. Another recessive factor, tassel seed, changes the staminate flowers in the terminal inflorescence to pistillate flowers.

The lateral inflorescences are unchanged. These plants are functionally females. Crossing pistillate plants by staminate plants gives normal hermaphrodites in the first generation. The usual mendelian recombination in the second generation produces normal hermaphrodites, pistillate, and staminate plants. The double recessives are pistillate plants of unexpected character and were not at first recognized; breeding tests proved their composition. Crossing these double recessive pistillate plants with staminate plants, homozygous for silkless but heterozygous for tassel seed, gives only pistillate and staminate plants in the following generations. A monosexual organism has thus changed to a bisexual organism under experimental control.

4. Hybridization of Tripsacum with Zea mays and Euchlaena mexicana. P. C. Mangelsdorf and R. G. Reeves, Texas Agricultural Experiment Station, College Station, Texas. (Lantern; 10 min.)

Tripsacum dactyloides is a relative of maize with a wide natural distribution in the United States. It has never been previously crossed with maize and no natural hybrids are known. The pollen of Tripsacum germinates readily on the styles of maize and the pollen tubes enter the stylar tissue. Fertilization does not occur, however, unless the maize styles are cut off to a length of about ½ inch, which corresponds to the style length of Tripsacum. When the styles are shortened, literally thousands of seeds can be produced. Most of these are abnormal in development and only a few mature. Cytological studies show that the chromosome number in both embryo and endosperm agrees with the number expected if the seeds are true hybrids. By shortening the styles it has also been possible to hybridize Tripsacum with Euchlaena mexicana, another genus of the same tribe, which is already known to cross readily with maize. These various intergeneric crosses have some bearing on the relationship of Zea mays to other species.

5. The meiotic phenomena in Drosophila melanogaster. Bessie B. League, University of Texas. (Introduced by T. S. Painter.) (Lantern; 8 min.)

This study of the male gonads of D. melanogaster was undertaken with the threefold object of determining the condition of the germ cells, of studying the behavior of the chromosomes during meiosis, and of clarifying the problem of the normality of the first maturation division. There are spermatogonia and spermatocytes in the early growth stage in larvae of thirty-six hours, but no maturation divisions have been observed until ninety-six hours. Thus, the chromosomes of larvae after thirty-six hours are no further advanced than the four-strand stage.

According to my interpretation, the behavior of the male germ cells in maturation is as follows: The telophase spermatogonial chromosomes form prochromosomes which give rise to leptotene threads, and these synapse and give place to the bivalent number of pachytene threads. The pachytene elements split and form the diplotene threads which become the diffuse masses of the early growth stage and then increase greatly in size to form the 'pretetrads' from which a considerable amount of plasmosome-like material is separated in the late growth period. The conjugated chromosomes condense into typical tetrads during dia-

kinesis and enter the division figure in normal fashion. This interpretation of meiosis in the male differs from other recent accounts in that synapsis does not occur during diakinesis, as some authors have maintained, but takes place at the normal time and is followed by typical pachytene and diplotene stages.

6. Chromosome numbers in the Cucurbitaceae. Thomas W. Whitaker, University of Virginia. (Introduced by Orland E. White.) (10 min.)

The taxonomic position and geographical distribution of the species of the Cucurbitaceae with known chromosome numbers are discussed from the viewpoint of their existing relationships. Chromosome size, shape, and stability in this family are also considered. A cytological study of peculiar forms of several cultivated species has so far failed to reveal any abnormal chromosome numbers or behavior. The results of chromosome counts of four species are presented (Luffa acutagula, Ecballium elaterium, Momordica balsamia, and Momoridica charantia).

7. The chromosomes of Clarkia. L. L. Burlingame, Stanford University. (Lantern; 15 min.)

The chromosomes of Clarkia species vary in number in the cells of the same plant with a range from four to twelve pairs. This is due to chance segmentation of the spireme. In consequence, these chromosomes do not possess specific individuality in respect to form, fiber attachment, size, or constrictions. The normal mean number is probably to be considered seven (pairs). Particular genetic lines may, however, show a mean number of eight or nine or possibly more. This appears to be due to two causes: non-disjunction and precocious separation of tetrads at M I. In some of these lines there is genetic evidence of factor duplication.

8. On the cytology of Matthiola incana in relation to the inheritance of double flowers. J. Philp and C. L. Huskins, McGill University. (15 min.)

Double-flowered stocks, Matthiola incana, are completely sterile. They are obtained from single-flowered strains which are of three distinct genetic types, 1) pure-breeding singles, 2) ordinary heterozygous singles which give 25 per cent double and 75 per cent single-flowered progeny, and, 3) eversporting singles which give about 53 per cent of doubles and 47 per cent singles. All the singles produced by eversporting singles continue to be of the eversporting type. This is the type which is of chief horticultural value. Its genetic behavior can best be explained on a lethal-factor hypothesis. Cytological study has shown that there is actually a minute fragment missing from one of the chromosomes and that the absence of this fragment constitutes the lethal factor. These observations have been confirmed by further study of plants with extra chromosomes, which were provided by Dr. H. B. Frost.

The study also provides evidence for Darlington's hypothesis showing the homology of mitosis and meiosis.

9. A cytological demonstration of the location of an interchange between two non-homologous chromosomes of Zea mays. Barbara McClintock, Cornell University. (Lantern; 15 min.)

A case of semisterility in maize was found to be due to an unequal interchange between the second- and third-smallest chromosomes. The second-smallest chromosome is distinguishable from the third-smallest by the relative lengths of its two arms, by its achromatic spindle-fiber attachment region, which is noticeably smaller, and, in, certain strains, by an obvious, deeply staining, knob-like body located at the end of the shorter arm. Examination of the chromosome complement of plants homozygous for the interchange showed, in place of the second- and third-smallest chromosomes, two chromosomes of new morphological character, the knobbed chromosome with its long arm much increased, and a short chromosome about the size of the smallest chromosome of the complement. In midprophase of meiosis, in plants heterozygous for the interchange, the intimate association of the homologous parts of the chromosomes involved produced a cross-shaped synaptic complex. The points of interchange were located at the center of the cross. By means of the knob on one normal and one interchange chromosome, the known relative lengths of the different chromosomes, and the spindle-fiber attachment regions, it was possible to identify and interpret each member of the synaptic complex. The interchange points were located near the midportion of the long arm of the second-smallest chromosome and a short distance from the spindle-fiber attachment region in the long arm of the third-smallest chromosome.

10. The occurrence of unpaired chromosomes in hybrids between varieties of Triticum vulgare. Lillian Hollingshead, Dominion Rust Research Laboratory, Winnipeg, Manitoba. (Introduced by E. B. Babcock.) (Lantern; 10 min.)

In F_1 hybrids between Garnet and Marquillo, two varieties of Triticum vulgare, many pollen mother cells contain unpaired chromosomes at the heterotypic metaphase. There are usually two univalent chromosomes, but one, or more than two, are to be found occasionally. Of 912 cells from six plants, 43.2 per cent showed univalents.

In the same season the parental varieties showed a few similar irregularities. Of 1240 cells from four Garnet plants, 5.8 per cent showed univalents. Of 1656 cells from four Marquillo plants, 10.1 per cent showed univalents. In both hybrids and parental varieties the frequencies of cells exhibiting univalents varied in counts from different plants, and sometimes even from different anthers of the same plant, to a degree not to be expected on the basis of random sampling. Consequently, the percentages must be considered as only approximate, but there is no doubt that univalents occur much more frequently in the hybrids.

To determine whether other hybrids involving Garnet and Marquillo show as many univalents, these two varieties were each crossed with several others. Only the hybrids with Red Fife have been examined. The frequency of univalents in Marquillo-Red Fife is considerably lower than in Marquillo-Garnet, but higher than in the parental varieties. Of 908 cells from six plants, 18.2 per cent showed univalents. Preliminary work on Garnet-Red Fife indicates a similar condition here, but more work must be done before the frequency of univalents in this hybrid can be even approximated.

Theories to account for the occurrence of univalents will not be discussed until more data are available. Their frequency in other varietal hybrids, and the extent of variation in frequency within varieties or hybrids and its connection with environmental or other conditions, are yet to be studied. The genetic significance of such irregularities, probably leading to the formation of gametes of abnormal chromosome constitutions, is obvious.

11. The frequency of mutation of specific genes in maize. L. J. Stadler, United States Department of Agriculture and University of Missouri. (Lantern; 15 min.)

Specific mutation rates have previously been determined only for genes known to be highly mutable. It is probable that the category 'gene mutation' includes fundamentally diverse phenomena, and consequently the analysis of such selected cases is a precarious basis for generalization. Although the quantitative study of mutation in unselected genes involves the genetic testing of extremely large numbers of gametes, it is feasible in maize in the case of genes for endosperm characters.

The frequency of recessive mutation was determined for eight genes of well-known and typical genetic behavior (*C, I, Pr, R, Sh, Su, Wx,* and *Y*). None of these had previously been known to mutate. The method used eliminates the possibility of error from contamination, and permits the testing of almost unlimited numbers of gametes.

Mutations were found for all of the genes tested except *Wx*, for which no mutants occurred among about two million gametes tested. For the other seven genes, the frequency of mutant gametes per million varied from about 400 for *R* to less than one for *Sh*. There was also distinct and consistent variation in the frequency of mutation of the same gene in different stocks.

X-ray treatments applied to the young ear shoots before megasporogenesis, in intensity sufficient to induce numerous recessive mutations for seedling characters, had no effect on the rate of mutation of *R*. The x-rayed ears yielded twenty-three mutants among 17,292 gametes tested, as compared with twenty-six among 21,865 gametes in the control. The genes *Pr, Su,* and *Sh* were also tested in this experiment, but because of their lower normal mutation rates the results are not conclusive. If the treatment had increased the rate of mutation one-hundred-fold, seventeen mutations of *Pr*, five of *Su*, and one of *Sh* would have been expected among the gametes tested. No mutations were found for these genes.

12. The relation between x-ray intensity and the frequency of deficiency in the maize endosperm. S. F. Goodsell, University of Missouri. (Introduced by L. J. Stadler.) (Lantern; 10 min.)

Deficiency induced by x-ray treatment is shown in heterozygous maize endosperms by the appearance of mosaic areas having the recessive character. The effect of x-ray dosage upon frequency of deficiency was determined by irradiating young ears forty-eight to fifty-four hours after pollination with seven graded doses ranging to the lethal limit (about 1000 r-units). The loci *A, C, Wx,* and *Su* were used most extensively, supplemented by *Pr, In,* and *Y* in smaller num-

bers. A total of 10,175 deficient areas were classified, including areas for all loci under investigation. For each locus the frequency of deficiency is directly proportional to intensity of the dose. Deficiencies for *A* occurred about three times as often as for *Su, In,* or *Pr* and about four times as often as for *C Wx.*

13. Some genetic effects of electromagnetic treatments in maize. George F. Sprague, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. (10 min.)

Electromagnetic treatments had an effect on the frequency of translocations and endosperm mosaics.

Plants were self-pollinated with pollen which had been exposed to an electromagnetic field. The plants grown from the resulting kernels were selfed. A few recessive mutations were observed.

Recessive stocks were pollinated with their corresponding dominants, the genes involved being *c, wx, su,* and *pr.* Ear shoots of such crosses were exposed between the poles of the magnet twenty-four or thirty-six hours after pollination. Exposures of fifteen, thirty, and forty-five minutes were used.

Endosperm mosaics were from two to three times as numerous in the treated ears as in the controls. The frequency of mosaics increased with an increase in duration of exposure, but not proportionally.

The percentage frequency of all mosaics was 0.54 in the controls and 1.57 and 1.18 in ears treated twenty-four and thirty-six hours after pollination, respectively.

The frequency of endosperm mosaics in both treated and control ears was markedly influenced by pollen from different genetic sources.

Back-cross and F_2 data indicate translocations or chromosome attachments involving the *R* and *Su* and also the *Y* and *Su* chromosomes. Other attachments were suggested, but not proved.

Certain F_2 ears, exposed to the magnetic field in the F_1 generation, exhibited ratios suggesting chromosome duplication.

14. Pollen-tube growth and seed production after radium treatment. J. T. Buchholz, University of Illinois, and A. F. Blakeslee, Carnegie Institution of Washington. (Lantern; 12 min.)

Various methods of treating pollen with radium are described, also the relation between dosage and the immediate physiological effect on pollen-tube growth and the relation between dosage and the proportion of seeds obtained when the pollen is used in pollinations of untreated females. Examples will be shown of genes giving inherited effects recognizable during pollen-tube growth, but which have no visible effects on heterozygous sporophytes. Many of the latter are transmitted through female gametophyte generation in 1:1 ratio and eliminated by gametophytic selection in the male gametophyte. Five different methods of this elimination during pollen-tube growth may be recognized from a study of the tests of pollen-tube distributions.

15. F_1's from radium-treated pollen. A. F. Blakeslee, S. Satina, A. D. Bergner, J. S. Potter, and A. G. Avery. (Lantern; 15 min.)

Of 400 F_1 individuals from pollen treated with radium for two to two and one-half hours (compare previous paper), nearly half (196) were distinctly abnormal in appearance. Shorter treatments are also included in the following tabulations. Of 209 normal-appearing plants, forty-one (20 per cent) had high percentages of aborted pollen; while, of 204 abnormal-appearing plants, 171 (84 per cent) had bad pollen. Chromosomal abnormalities include segmental interchanges causing closed circles of four to six members; simple translocations causing chains of four or more members; deficiencies for a whole or part of a chromosome; additions of a chromosome or fragment. Of 187 normal-appearing plants examined cytologically, thirty (16 per cent) showed chromosomal abnormalities. Of eighty-two abnormal-appearing plants, sixty (73 per cent) showed chromosomal abnormalities. Treated pollen introduced abnormalities incapable of transmission through pollen in later generation. These probably were protected by normal pollen-tube nucleus. Possibly in plants, as in animals, no gamete lethals exist, though gametophytic lethals are common. So far in this series, thirty-eight circles and thirty-one chains have been discovered. From these heterozygous individuals, it may be possible by inbreeding to isolate 'prime types' (forms homozygous for the modified chromosomes) for use as chromosomal testers. Individuals with circles are mostly normal in appearance and mostly have good pollen. Those with chains are mostly abnormal in appearance and generally have bad pollen. Increase in time of treatment from two to four and one-half hours failed to result in an orderly increase in chromosomal abnormalities, aborted pollen, or obvious abnormalities in appearance.

16. The effect of ultraviolet radiation on mutation. Edgar Altenburg, The Rice Institute. (15 min.)

In a previous paper I reported that mixed ultraviolet light was either non-effective or at least far less effective than x-rays in producing mutations in Drosophila. Methods have now been used permitting a heavier dose to reach the germ cells without sterilizing. When the radiation intensity was lessened, a disproportionately longer duration of it was found to be endurable. Stocks of yellow white and yellow eosin, having less pigmentation on abdomen and testes, were used, to obtain stronger treatment of germ cells without correspondingly more physiological damage. In one series eggs and larvae were used, because of their greater transparency.

The source of radiation was a quartz-mercury arc lamp of 4 to 5 amperes and 60 to 90 volts. Distance of flies from lamp was 3 meters. Time was one-half to forty-four hours. Only sex-linked mutations were looked for, as previously. The genetic analysis was done according to Muller's 'ClB' method.

In the 'adult treated' experiment, a count of 1020 tested daughters gave nine with lethals (all 'point mutations') in their treated X chromosomes. In the 'eggs and larvae treated' experiment, 279 tested daughters gave three with lethals. In the controls (lumped together from both experiments) 643 tested daughters gave no lethals. These results indicate a positive effect of ultraviolet light on mutation frequency, even though an effect of a lower order than from x-rays of equally deleterious dosage. Tests of the nature of the mutations and trials of individual wave lengths, now projected, should be of considerable theoretical interest.

17. Disorganization of wing veins caused by x-raying larvae of Habrobracon. Anna R. Whiting, Pennsylvania College for Women. (Lantern; 10 min.)

Progeny from type stock (no. 31) and from cross of ivory reduced females (no. 27) by type males (no. 31) were first used. Offspring of each mother were divided into two separate gelatine capsules and used as controls and treated. (K. V. P. 50, ma. 8, target distance 15 cm., Al. shield $\frac{1}{2}$ mm., time of exposure, 15, 20, 25, and 30 minutes.) Series *a* consisted of larvae forty-eight to ninety-six hours old when rayed; series *b*, eggs, and larvae one to forty-eight hours old. Lower mortality of immature and adult wasps and less sterility occurred in series *a*. A high percentage of all treated individuals showed non-inherited defects of antennae, eyes, wings, legs, etc. Many treated heterozygous (Rr) females, the majority from series *a*, showed veins badly disorganized, 'shot,' resembling reduced (r). Percentage varied (11.361 to 31.428 per cent) significantly with duration of treatment. A few (0.476 to 6.450 per cent) treated type individuals were slightly 'shot.' 13,038 controls were normal.

Larvae from another type stock (no. 1) and from cross of no. 27 females by no. 1 males (including in the latter case some 'diploid' males) were then used (series *a*, controls and treated, twenty-five minutes).

A significantly higher percentage of 'shot' individuals occurred in no. 1 than in no. 31. In both groups veins were but slightly 'shot.' Among both kinds of treated heterozygous females and among 'diploid' males 'shot' occurred in similar percentages and with extreme disorganization. 'Shot' no. 1 females occurred in percentage similar to heterozygous females.

18. A study of mutations, mosaics, and non-inherited abnormalities among progeny of individuals x-rayed as larvae in Habrobracon. Anna R. Whiting, Pennsylvania College for Women, and Carey H. Bostian, North Carolina State College. (Lantern; 5 min.)

One hundred and seventy-four females treated with x-rays as described in previous abstract were set. These were taken from type stock no. 31 and from cross of stock no. 27 females by no. 31 males, series *a* and *b*. One hundred and fifteen control sisters were likewise set. One hundred and two of former group, but only nine of latter, died without progeny. 17,078 offspring of surviving treated and control females were studied for mutations, physical abnormalities, and mosaics. Data from series *a* and *b* and from the various treatments are combined, since they are not significantly different. Among 1741 sons of treated heterozygous females appeared five mosaics for eye and wing characters. Among 8257 sons of heterozygous controls appeared six similar mosaics. (Mosaics of this type are produced only by heterozygous females.) Mutations occurred in two of 3999 sons of treated, in one of 11,990 sons of controls. Abnormal types not inherited (occurring in males and females) were six in 4040 progeny of treated, eight in 13,038 controls. Differences between percentages in rayed and controls are suggestive, although not statistically significant. A few heterozygous females were treated for twenty minutes as five-day larvae or prepupae. Among their 576 sons appeared one mutation and two mosaics.

19. Evidence of the divisibility of the gene. I. J. Agol, Timiriazef Biological Institute, Moscow, and University of Texas. (Introduced by H. J. Muller.) (15 min.)

1. Evidence of gene divisibility was first obtained by the Moscow geneticists in the phenomenon of partial ('step') allelomorphism of scute. Scute consists of subgenes in linear order, different blocks of which mutate to form different scute allelomorphs. The method of investigation used involved study of compounds, in lieu of crossing-over. The success of Dubinin (Moscow) in obtaining, by successive mutations, two scute genes in one chromosome illustrates this interpretation strikingly.

2. We have now obtained still more striking evidence of the linear divisibility of scute in the laboratory of Professor Muller, using a method involving deletions. We covered with a deleted X-chromosome a block of subgenes in the left-hand region of scute, leaving the right-hand region uncovered.

3. We have studied five allelomorphs of lozenge. Though qualitatively similar, they too are partial allelomorphs, since their compounds are often phaenotypically more normal than the homozygotes. A provisional map for lozenge, like that for scute, has been constructed.

4. Our preliminary investigations indicate that miniature and dusky also are partially allelomorphic. This is noteworthy because of their occasional recombination by crossing-over.

5. Evidence indicating partial allelomorphism is found on examination of already reported data concerning the series involving Gull and other 'deficiencies' of Mohr and Bridges, spineless, eyeless, and perhaps truncate.

6. All this convergent evidence gives us the right to generalize the above theory of gene structure, and to substitute it for the theory of genes as indivisible beads. We are thus compelled to reconsider, from the new viewpoint, many facts and theories of modern genetics.

20. Complex gene rearrangements induced by x-rays. C. P. Oliver, Washington University. (Introduced by Caswell Grave.) (Lantern; 15 min.)

Analysis of various translocations in Drosophila indicates that instead of being simple translocations they often involve several simultaneous breaks and reattachments. In one case of this kind, a piece deleted out of the third chromosome has been inserted into the X-chromosome in a non-terminal position. III was broken between curled and stripe and also between ebony and claret, the section between those two breaks being attached to X near echinus. Presumably the X-chromosome became broken at this point to allow the insertion. Cytological preparations show a shortened III and a lengthened X, but no indication of a branched structure. Crossing-over occurs between the shortened III and a normal III on either side of the fracture, and between the lengthened X and a normal X on both sides of the insertion, but is characteristically affected in frequency. Hyperploids are viable and breed. In a second case a section became deleted from the X-chromosome and attached to chromosome III. The two ends of X reunited. In each of two other cases, a large section of the left half of III has become attached to the right end of II. An inversion, not extending to the end, has occurred at the same time in the right half of II. In both cases there is a dominant phaenotypic effect upon the eye color, in one case uniform, in the other case variegated. The genetic interpretation of these two translocations has been checked cytologically.

21. An egg lethal associated with the pervirens factor in Oenothera. George H. Shull, Princeton University. (Lantern; 15 min.)

In reporting the first case of linkage in the third linkage group in Oenothera, it was pointed out that double-flowered old-golds (vs_p) segregated from their single-flowered yellow F_1 hybrids as the recessive type in a typical monohybrid mendelian ratio, but that, on the other hand, all of the offspring in the same segregating family had rubricalyx buds, notwithstanding the fact that the parent of the given progeny had been likewise heterozygous for the green buds and green stems of mut. pervirens. This failure of the pervirens character to reappear in this F_2 family was attributed to the presence of one of the Lamarckiana zygote lethals in association with the pervirens factor. Subsequent tests of this proposition have shown the unanticipated presence of a gamete lethal instead of, or perhaps in addition to, the zygote lethals. No gamete lethals have been known heretofore in Oe. Lamarckiana or its direct derivatives, although they are quite frequently present in other species of Oenothera.

22. Cytological evidence of genetical relationships in Oenothera. Ralph E. Cleland, Goucher College. (15 min.)

It is now fairly evident that a close correlation exists between chromosome configuration and genetical relationship in Oenothera. The following statements seem to be in general true: 1) Plants with identical genetical make-up have in general identical chromosome configurations. 2) Complexes from different races which are identical genetically give identical chromosome configurations with a third complex, and those which are closely related give similar configurations. 3) Complexes which are genetically closely related to each other yield, when combined, configurations in which most of the chromosomes are paired. Those which are distantly related give configurations in which most or all of the chromosomes are involved in circles. The extent to which the truth of these statements has been tested is indicated.

23. The genetics and cytology of a tetraploid sport from Oenothera franciscana. B. M. Davis, University of Michigan. (Lantern; 15 min.)

A tetraploid of Oenothera franciscana has arisen by way of a triploid plant in the F_2 of the cross franciscana (YYTT) $\times$ franciscana sulfurea dwarf (yytt). The triploid, one of four similar plants, must have arisen through the union of a seven-chromosome franciscana gamete (YT) with a fourteen-chromosome gamete (YTYT), the latter resulting from the suppression of the homoeotypic mitosis and consequent inclusion of two sets of franciscana chromosomes in a restitution nucleus. The triploid gave a progeny of franciscana together with various dwarfs and three tetraploids. A line from one of the tetraploids has bred true through three generations except for occasional dwarfs that fail to mature.

The twenty-one chromosomes of the triploid emerge from second contraction in short chains, in pairs, and in trisomes. If seven trisomes are present, they may lie so that seven chromosomes pass to one pole of the heterotypic spindle and fourteen to the other, but such segregation is not common. Generally the

segregation is irregular, which accounts for the 60 per cent of shriveled pollen grains and the low seed germination of 22 per cent. The tetraploid results from the union of fourteen-chromosome gametes.

The twenty-eight chromosomes of the tetraploid are found mostly paired at metaphase of the heterotypic mitosis, but trisomes and unpaired chromosomes are also present. The latter are responsible for much irregularity in segregation, which explains the 80 per cent of shriveled pollen grains. A regular segregation at meiosis gives gametes with fourteen chromosomes which reproduce the tetraploid.

24. The relation of size to shape in the heredity and development of Cucurbita fruits. Edmund W. Sinnott, Barnard College, Columbia University. (Lantern; 10 min.)

Shape of fruit in Cucurbita pepo has been shown to be controlled by a series of mendelian factors. In cases where a single factor has a marked effect and two segregating shape groups can be distinguished sharply in F_2, these groups are found to be essentially similar in size, regardless of size differences between the original parents, thus indicating that size factors and shape factors are inherited independently. In more complex cases there is also little or no correlation between size and shape in the F_2. In certain lines, particularly those with flat or disk fruits, the size attained by the fruit at maturity was found to affect to some extent its shape, the larger fruits being relatively somewhat flatter than the smaller ones. Developmental studies of female flowers and young fruits showed that from pollination to ripe fruit there is a progressive increase in the amount of flattening. Thus there is a positive correlation between size and shape in development, but an entire absence of correlation between these two traits in inheritance.

25. Genetic control of susceptibility to the inoculated mouse leukemias of lines I and L. E. C. MacDowell, Department of Genetics, Carnegie Institution, and M. N. Richter, Department of Pathology, College of Physicians and Surgeons. (Lantern; 15 min.)

As previously reported, the lymphatic leukemia originating in a spontaneous case in strain C58 may be propagated continuously by successive inoculations into young mice of the same strain. All the mice of this strain appear to provide the necessary conditions for the survival of the active agent and hence, with rare exceptions, die shortly after the inoculation. However, the requirements for survival of the leukemia in inoculation *lines* from different spontaneous cases are not necessarily the same. Two of the *lines* when inoculated into mice of a backcross with a resistant strain find suitable conditions for their growth in only small numbers of the mice; a third line (line I) inoculated into mice of the same cross gives a 1: 1 ratio. The present data verify this ratio.

Among several other strains of mice tested with line I only one, strain 89, has given any susceptible mice. In this case more than half the mice are susceptible.

From a spontaneous case in strain 89, a line of inoculated leukemia has been established (line L) in young mice of the same strain, 89, all of which appear to provide the necessary environment for the growth of this leukemia. However, none of the mice of strain C58 has been found to be susceptible to the leukemia of line L.

26. Differential reaction in disease resistance within two highly inbred strains of rats and the reaction of their hybrid progeny. M. R. Irwin, The University of Wisconsin. (15 min.)

Descendants of the Wistar 'A' and 'B' strains have produced deviations from the uniformity in percentage mortality expected in such highly inbred strains. Within the 'A' strain, at least, the majority of the reactions apart from the expected have appeared in a particular line within the strain and within particular litters. No suggestion as to a change of the pathogenicity of the infecting agent (a strain of S. enteritidis) has been encountered which could account for the differences observed. A specific dose of the organism has been used throughout the experiment, with adequate and uniformly reacting controls.

Contributing evidence is given in the reactions of hybrids between these two strains of animals. Four female progeny from a brother-sister (survivor) mating of the 'B' stock were mated with two males (brothers) of the 'A' strain. The progeny from these matings showed a significant increase in the number of resistant individuals over that of the average of either parental strain; thirty-seven individuals at 32 per cent mortality, as compared with 94 and 81 per cent mortality, respectively, for the 'A' and 'B' strains. The back-crossed progeny (to the 'A' strain) and F_2 progeny from surviving individuals in the F_1 stock gave a mortality of 53 and 22 per cent for sixty-two and fifty-five individuals, respectively.

While heterosis might explain in part the increased resistance of the F_1 progeny over that of the parental stocks, no evidence of heterosis as a factor in resistance was obtained in the reaction of hybrid progeny from each parental strain with other and different stocks, when subjected to the same test.

27. A critique of the various hypotheses of the inheritance of the blood groups. Laurence H. Snyder, Ohio State University. (Charts; 15 min.)

There are at present five hypotheses which have been offered to explain the heredity of the blood groups. Each of these has its advocates. The hypotheses are: 1) two independent pairs of factors, 2) two completely linked pairs of factors, 3) two partially linked pairs of factors, 4) triple allelomorphs, 5) four allelomorphs. These are critically reviewed on the basis of, 1) mass statistics and, 2) the author's own family histories in conjunction with those of other investigators. On these bases the triple-allelomorph hypothesis appears to be the only one which can successfully stand. It is thus re-upheld that the blood groups are inherited as three multiple allelomorphs, the exceptions, if any really exist, being explainable on the basis of non-disjunction, as Levine has suggested. Thus the laws presented by the present author during the last three years, involving the various legal aspects of blood grouping, the linkage relations of the groups, and serologic race classification, are reaffirmed as valid laws.

28. The phenotype expression of the mutant, squatty, in Drosophila hydei. W. P. Spencer, College of Wooster. (Charts; 10 min.)

Squatty, a recessive mutant at locus 50 in the fourth chromosome of Drosophila hydei, is marked by a number of striking phenotypic characters. These include shortened body, broad, short wings with cross-veins close together, large eyes, and

thick, short legs. In addition to the above characters which are always present in homozygous individuals, other highly variable characters appear, including changes in the posterior cross-vein of the wing. The variation in this vein ranges from a condition in which the vein is complete to one in which it is entirely missing. Ten grades of character expression have been recognized. A study of the symmetrical correlation between the right and left wings in some 3000 individuals has made it possible to arrange the grades of character expression in a continuous series. It is shown, for instance, that the condition in which the medial half of the cross-vein is missing is a less extreme departure from the normal than the loss of the lateral half of the cross-vein. A study of the variable expression of genetic factors in terms of symmetry in bilaterally symmetrical organisms should lead to a better understanding of the nature of gene expression.

29. A generalized theory of four-strand crossing over. Alexander Weinstein, The Johns Hopkins University. (15 min.)

The formula for four-strand crossing over reported by the author (The Anatomical Record, vol. 41, pp. 109–110) has been applied to further experimental data, and the results confirm the conclusions previously arrived at.

These calculations throw light on the method by which interchange between chromosomes takes place; and they lead to a new formula embodying a generalized theory of four-strand crossing over.

30. A triple allelomorph in doves and its interspecific transfer. L. J. Cole, University of Wisconsin. (Lantern; 15 min.)

The normal dark color of certain wild doves is allelomorphic to the sex-linked 'blond' and 'white' allelomorphs of the domestic ring dove (Streptopelia risoria). By crossing with Spilopelia chinensis and backcrossing, the dark of the wild species has been transferred to birds that are now fifteen-sixteenths ring dove, thus making substantially a new variety of the domesticated dove. Similar crosses are being made, bringing in the dark color from the wild turtle dove (Turtur turtur). These have, however, as yet progressed only to the first backcross.

31. Further studies on inheritance in Cladocera. A. M. Banta and Thelma R. Wood, Carnegie Institution and Brown University. (Lantern; 15 min.)

The earlier studies on the occurrence of mutations in Daphnia longispina in parthenogenetic reproduction have been supplemented by further studies on inheritance in sexual reproduction. These show that after a clone has passed through a large number of parthenogenetic generations the fertilized eggs (derived from inbreeding among the members of the clone) differ markedly: 1) they have a lower hatchability; 2) the hatched offspring show lower viability and, 3) a much larger percentage of them is sterile; 4) they vary more in their productivity; and, 5) they exhibit a much wider variability in their rates of growth, etc., as compared with the sexually produced eggs and hatched offspring derived from inbreeding within a clone which has passed through a very limited number of parthenogenetic generations. The assumption is that recessive lethal, sublethal, and other mutations occur during parthenogenesis, accumulate as

parthenogenesis proceeds, and reveal their existence only when the clone is inbred in sexual reproduction. Other evidence derived from the continued breeding of Daphnia longispina is consistent with this assumption.

The great variability of the sexually produced offspring derived from inbreeding a clone long parthenogenetic—as contrasted with the relative uniformity (physiological and morphological) of the parthenogenetic offspring within a clone—becomes increasingly manifest as these studies continue.

32. The production of high lens-antibody content in rabbits. Heman L. Ibsen and L. D. Bushnell, Kansas State Agricultural College. (Lantern; 15 min.)

Guyer has employed three distinct methods of introducing lens antibody into rabbits. 1) Lens material was injected intraperitoneally into hens, thereby producing lens antibody which was subsequently injected in small doses of the hen serum into pregnant rabbits. 2) The lenses of the rabbits were needled, thus allowing the animals to produce their own antibody. Neither of these methods caused much antibody to be present in the blood of the rabbit. 3) Rabbit lens material was injected directly into the rabbit. Only a few animals were treated and were not tested afterward for their antibody content.

We have used a modification of the third method, and injected calf lenses instead of rabbit lenses. Six males and eleven females were injected intraperitoneally five times at weekly intervals with lens material equivalent to 1 gram of moist lens per 2500 grams' live weight of the treated animals. (The average weight of the rabbits was approximately 2500 grams.) Ten days after the last injection, blood was drawn and the serum tested for antibody. Both calf and rabbit lenses were used as antigens with no marked differences in results. The sera of all seventeen caused some precipitation when the lens material was diluted one part in 320, and there were eight in which lens precipitins were still effective when the dilution was one in 10,240. Unfortunately, nine of the eleven females died of coccidiosis before reproducing and the other two refused to take care of their litters during extremely hot weather. A new series is being injected subcutaneously.

33. The hereditary basis for melanosis in hybrid killifishes. Myron Gordon, Zoölogical Laboratory, Cornell University. (Introduced by H. D. Reed.)

An analysis of the results of both fish fanciers and geneticists reveals the fact that whenever the Pulchra (spotted, stippled), Rubra (spotted, stippled, red) and, under certain conditions, Nigra (spots in rows, stippled) varieties of Platypoecilus are used in generic crosses with Xiphophorus, melanotic tumors develop in some of the hybrid offspring.

On the other hand, when varieties of Platypoecilus, such as Stippled, One-spot, Twin-spot, and Crescent, are used in generic crosses with Xiphophorus, no comparable abnormal growth appears.

By genetical tests and morphological studies of the pigmentary equipment of pure strains it has been possible to show that all those varieties of Platypoecilus which give rise to the development of melanotic tumors in their hybrids possess macromelanophores, while those which merely possess micromelanophores give rise to normal offspring.

Previous work of the author has shown (Anat. Rec., 1926) that macromelanophore factor is sex-linked (Sp for spots), while the micromelanophore factor is autosomal (St for stipples).

In a crucial test, a Platypoecilus possessing the macromelanophores only, Sp sp (without micromelanophores, st st), was crossed with Xiphophorus with the result that melanotic tumors developed only in hybrids which possessed macromelanophores, Sp; other hybrids of the same brood but which did not possess macromelanophores, sp, were normal.

By demonstration

34. Species crosses in Aquilegia. Edgar Anderson, Missouri Botanical Garden and Washington University.

The genus Aquilegia is characterized by interspecific fertility. The most diverse American, Asiatic, and European species can be readily crossed and produce hybrids which are at least partially fertile. The genus is divided into four well-marked sections, whose phylogenetic relationships can, therefore, be studied experimentally by making crosses between sections. Preliminary results indicate certain regularities of dominance in interspecific hybrids. Midlength spurs are dominant to long spurs or to very short spurs. Short and wide laminae are dominant to long narrow ones.

35. Factor interactions in Primula sinensis. Edgar Anderson, Missouri Botanical Garden and Washington University.

A comparison of the effect of several mutant factors (chiefly those affecting leaf shape) on different genetic backgrounds. The dominant mutant 'sinensis' is contrasted with the recessive mutants 'oak,' 'fern,' etc.

36. Genetics herbarium. Edgar Anderson, Missouri Botanical Garden and Washington University.

Herbarium specimens and photographs illustrating genetical and cytological work, chiefly from the John Innes Horticultural Institution.

37. Chromosome studies in the genus Sorghum. C. L. Huskins and S. G. Smith, McGill University.

A cytological study has been made of twenty-four species and varieties of Sorghum, from the collection of the Royal Botanic Gardens, Kew. All except one species have twenty chromosomes, diploid. S. halepense has forty. One pair of peculiar-shaped chromosomes has been traced through all the species and in some possibly related genera. Certain peculiarities of chromosome behavior at meiosis seem to have significance regarding the origin of Sorghum and other genera. Tetraploid and octaploid segments were found in root tips of diploid species, and in one instance the cause of the tetraploidy is indicated.

38. Microscopic demonstration of the chromosomes of Clarkia. L. I. Burlingame, Stanford University.

39. Microscopic demonstration of cytological evidence of genetical relationships in Oenothera. Ralph E. Cleland, Goucher College.

40. New mutations in Habrobracon. Kathryn A. Gilmore, University of Pittsburgh. (Introduced by P. W. Whiting.)

The morphological characteristics of various mutants are being studied. Drawings and specimens will be demonstrated.

41. Translocations, deletions, and breakage in Drosophila melanogaster. T. S. Painter, University of Texas.

The frequent occurrence of breaks in chromosomes accompanied by translocations, deletions, and other morphological changes following irradiation with x-rays is of very great interest to biologists not only because it places in the hands of the geneticist newly made tools for the solution of many problems, but also because it is apparent that many of the changes in chromosome morphology during speciation can be explained on these grounds. This suggests that such changes may have played a considerable rôle in speciation in addition to point mutations in that such changes in chromosome morphology would lead to an effective sort of physiological isolation of the individuals bearing them. Some of the more striking translocations, deletions, and duplications found at this laboratory will be demonstrated.

42. A lethal defect in development of the chick embryo. T. C. Byerly and Morley A. Jull, Bureau of Animal Industry, U. S. Department of Agriculture.

Bones of affected embryos are so soft at hatching time that they prevent pipping; allantoic and amniotic fluids are unabsorbed; unabsorbed yolk is about twice normal amount; edema is usual. The defect was discovered in progenies from a mating of F_1 individuals from a Rhode Island Red ♂ × Barred Rock ♀'s. It is apparently inherited as a simple recessive. Five half-sisters and two less closely related F_1 ♀'s, mated to a half-brother of the half-sisters, produced 242 normal and 74 abnormal embryos that lived to the eighteenth day of incubation. Four F_1 ♀'s not closely related to the ♂ gave 149 normal chicks. The mother of an F_1 ♀ producing abnormals gave 13 normal chicks mated to a Barred Rock cockerel; 13 normal and 5 defective mated to the F_1 ♂ described.

Seven F_2 pullets, in back-cross, and half-brother × half-sister matings, produced 40 normal, 11 defective, and 5 doubtful chicks; 5 others, 29 normal and 10 doubtful; 16 others, 89 normal chicks. 'Doubtful' chicks had fairly sound bones, but had failed to absorb allantoic and amniotic fluids. More data are necessary before concluding finally that the defect described is inherited as a simple mendelian recessive, lethal in homozygous condition, or is not. Present data indicate no linkage with down color.

By title

43. Genetic results from conjugation of double monsters and free individuals of Paramecium caudatum. Charles F. DeGaris, Department of Anatomy, Johns Hopkins University.

In ten pure lines of Paramecium caudatum fission rates and mean cell lengths were ascertained over a period of eighty-five days. Then double monsters were produced in each line by cyanide vapor or by reduced temperature. These double monsters were allowed to conjugate with individuals either of their own lines or of other lines, and either by anterior component or posterior component, or both. Then resulting free progeny of double monsters and respective free conjugants were used to start new pure lines, whose fission rates and mean cell lengths were ascertained. It has previously been shown that the experience of monster formation alone does not alter fission rates and mean cell lengths. It is shown from the above experiments that conjugation of monsters does alter the fission rates, or mean cell lengths, or both in more than three-fourths of the lines examined.

44. Nucleus versus cytoplasm in the heredity of Paramecium caudatum as shown by conjugation of double monsters. Charles F. DeGaris, Department of Anatomy, Johns Hopkins University.

Since in the double monsters of the foregoing experiments there was a free exchange of considerable parts of the cytoplasm by cyclosis from anterior component to posterior component and vice versa, and since free progeny of these two components, after conjugation of the monsters, often showed marked differences in fission rate, or mean cell length, or both, it seems necessary to refer such diversities to preceding diversities—in this case, to diversities arising in the nuclei as a result of conjugation. Thus within a cytoplasm most of which was common to the two components, but with nuclei rendered diverse by conjugation, heritable differences arose in more than three-fourths of the lines. The few lines not showing differences after conjugation may have come from double monsters, which, though apparently conjugating with free individuals, failed to complete the act. Or if the act were complete, there is the further possibility that in some lines conjugation might result in new combinations which would allow the fission rate, or the mean cell length, or both, to remain unaltered.

45. Analysis of several induced gene rearrangements involving the X-chromosome of Drosophila. H. J. Muller and W. S. Stone, University of Texas.

1. The rearranged X-chromosome termed 'delta-49' undergoes practically no crossing-over except near the right end, but considerable non-disjunction, with a normal X, and much crossing-over but little non-disjunction with another 'delta-49.' For analysis, it was repeatedly x-rayed. Known genes (or allelomorphs) so obtained were found to have the linkage arrangement: sc-pn-w-ec-rb-*fw-m-lz-cmn*-f. Genes italicized have inverted arrangement. Cross-over frequencies are as expected for this arrangement.

2. A 'C1B' chromosome, on irradiation, became partially reinverted, so that it again crossed over freely with normal X's. Cross-overs having their left end

from normal, right end from reinverted X, are phaenotypically unaffected, but contain, inserted in right region, a duplication of loci *sh* and *ct*, derived from left region. Hence both breakages of reinversion were to left of respective 'C1B' breakages. Left breakages on both occasions were between *cv* and *sn*; right breakages are being located. Lethal of 'C1B' arose at left breakage only. Effects of insertion on crossing-over are being studied.

3. A phaenotypically unmodified X-chromosome showing reduced crossing-over with normal proves to have a section translocated to the fourth ('translocation X⟶IV 1'). This section was non-terminal, but the left and right remainders became reunited, forming a deleted X-chromosome. Left break was between *lz* and *v*, right between *car* and terminus. Zygotes with one entire X plus either fragment die, but females or 'superfemales' with two entire X's plus either fragment live and breed. Right fragment 'dilutes' Bar. Position of sex differentiator(s?) is being studied through crosses of this stock to triploids and resultant production of 'intersex-plus' individuals.

46. Mutations and freaks from x-rayed adult males of Habrobracon. R. Elizabeth Chalmers, University of Pittsburgh. (Introduced by P. W. Whiting.)

Freshly eclosed males of inbred stock (no. 11) were treated (fifteen to twenty-five minutes at 45 KVP and eight minutes at 76 KVP, target distance 15 cm., ma. 8, Al. shield $\frac{1}{2}$ mm.) and mated to females of same stock. Among 877 F_1 females (50 per cent treated germplasm) were seven freaks (0.7980 per cent), while among 3418 F_2 females (25 per cent treated germplasm) were twenty-nine freaks (0.8484 per cent). Among 3338 F_1 males (untreated germplasm) were fifteen freaks (0.4494 per cent), while among 5328 F_2 males (50 per cent treated germplasm) were sixty-six freaks (1.2387 per cent). The difference in rate of occurrence of freaks among F_1 and F_2 males (0.7893 per cent $\pm$ 0.1908 S.E.) indicates possible influence of treatment increasing hereditary tendency toward production of abnormalities.

None of the 162 mates of treated males proved heterozygous for a visible mutation. Four new visibles appeared in F_2. Of the 654 tested daughters of treated males, three, sired by different males, carried *narrow*, *twisted*, and *semi-long*, respectively. Of eleven tested daughters of one treated male, four, sired 26+ days after treatment, were heterozygous for *small-wing*, indicating that mutation had occurred at time of treatment.

The fact that no mutations were observed among 4293 carefully examined F_1 males or as single individuals among 8154 F_2 males indicates that visible mutation did not take place in the 12,447 eggs from which these males developed.

47. The production of mosaics in Drosophila by x-rays. J. T. Patterson, University of Texas.

A study on the effect of x-raying the germ cells of Drosophila melanogaster in the production of mosaics, including sex mosaics or gynandromorphs. It was found that treating the germ cells of one of the parents increased the frequency of the appearance of gynandromorphs about three times over that found in the untreated or control material. X-raying the X-bearing sperm cells results almost exclusively in the elimination of the treated X-chromosome from the male parts

of the gynandromorphs, whereas treating the egg cells results in the elimination of the treated and untreated X-chromosomes with about the same frequency. Gynandromorphs can be produced by breaking the X-chromosome by irradiation. Such mosaics have in the male parts an extra fragment of an X-chromosome, in addition to one entire X-chromosome. Advantage may be taken of the effects of breaks to study the question of a possible sex factor in the X-chromosome. An analysis of gynandromorphs and aberrant males and females produced by breakage indicates that if there is a gene for sex, it must lie at some point on the chromosome between the loci for singed and forked or their normal allelomorphs.

48. On the occurrence of so-called sex chromosomes in hermaphrodite animals. E. C. Jeffrey, Harvard University.

In the last edition of his classic work on "The cell in development and heredity" Wilson remarks that the examination of hermaphrodite animals for structures resembling sex chromosomes would constitute the crucial evidence in regard to their validity. The huge phylum of worms constitutes absolutely the best material in this connection, for in it the species are not only numerous, but are very generally hermaphrodite. A study of three large groups of the Vermes has shown that lagging chromosomes of the type generally designated by zoölogists as sex chromosomes are commonly present in worms. It is of interest to note in this respect that these chromosomes which lag in meiosis are of the univalent type and consequently correspond to the XO or to the multiple sex-chromosome conditions. It would appear, accordingly, that so-called sex chromosomes of the univalent type are open to serious doubt as indicators of sex. Their real significance apparently is as supplying evidence of the hybrid origin of parthenogenetic forms, since in practically all the cases studied parthenogenesis and hermaphroditism occur together.

49. Melanotic tumors in certain hybrids of Mexican killifishes. H. D. Reed, Zoölogical Laboratory, Cornell University.

When the proper crosses of Mexican killifishes are made, certain of the offspring are affected with differing degrees of melanosis which involves a large type of black pigment cells, called by Gordon macromelanophores. This 'malady' is expressed in three different degrees or states: 1) an abnormal invasion of the skin by macromelanophores which may extend over the whole surface of the body and fins; 2) an intense invasion of a given area from the corium inward (here called general melanosis) accompanied by deterioration of affected parts and, in extreme cases, resulting in the loss of scales and fin rays and a final replacement of normal tissues by macromelanophores; 3) the appearance of pronounced and sharply delineated tumors in which the overgrowth is associated with a true neoplasm.

AMERICAN SOCIETY OF ZOÖLOGISTS

CONSTITUTION, OFFICERS, AND MEETING PLACES OF THE SOCIETY

CONSTITUTION

Article I

NAME AND OBJECT

Section 1. The Society shall be called the "American Society of Zoölogists."

Sec. 2. The object of the Society shall be the association of workers in the field of Zoölogy for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoölogical investigation in this country.

Article II

MEMBERSHIP

Section 1. Members of the Society shall be elected from persons who are active workers in the field of Zoölogy and who have contributed to the advancement of that science.

Zoölogists without the training in research required for full membership may be elected associate members of the Society.

Sec. 2. Election to membership in the Society shall be upon recommendation of the Executive Committee.

Sec. 3. Each member shall pay to the Treasurer an annual assessment as determined by the Society. This assessment shall be considered due at the annual meeting and the name of any member two years in arrears for annual assessments shall be erased from the list of members of the Society, and no such person shall be restored to membership unless his arrearages shall have been paid or he shall have been re-elected.

Sec. 4. Foreign Zoölogists, not members of this Society, may be elected Honorary Fellows upon unanimous recommendation of the Executive Committee by a majority vote of the members present at any meeting of the Society. Honorary Fellows shall not be required to pay dues.

Article III

OFFICERS

Section 1. The officers of the Society shall be a President, a Vice-President, a Secretary and a Treasurer and the members at large of the Executive Committee.

Sec. 2. The Executive Committee shall consist of the President, the Vice-President, the Secretary, the Treasurer and five members elected from the Society at

large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve out the remainder of his term.

Sec. 3. These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

Sec. 4. The officers named in Section 1 shall discharge the duties usually assigned to their respective offices.

Sec. 5. Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled by appointment, until the next annual meeting, by the Executive Committee.

Sec. 6. At the annual meeting the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than one month before the next annual meeting. It shall be the duty of the Secretary to mail the list of nominations to all members of the Society at least two weeks before the annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

Article IV

meetings of the society

Section 1. Unless previously determined by the Society the time and place of the annual meeting of the Society shall be determined by its Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

Sec. 2. Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

Article V

quorum

Twenty-five members shall constitute a quorum of the Society and four a quorum of its Executive Committee.

Article VI

changes in the constitution

Amendments to this Constitution may be adopted at any meeting of the Society by a two-thirds vote of the members present, upon the following conditions:

(a) The proposed amendment must be in writing and signed by at least five members of the Society.

(b) This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

(c) The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

BY-LAWS

DUES

(1) The annual dues for members or associate members, unless remitted or changed by the vote of the Society, shall be two dollars.

SECRETARY

(2) The duties and privileges of the Secretary shall be as follows:

(a) He shall keep the records of the Society.

(b) Whenever the proper officers of a number of related societies shall have a conference with a view of determining a common time and place for the several annual meetings, he shall act as the delegate or representative of this Society. (See also 5.)

(c) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society, and

(d) He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

TREASURER

(3) The duties and privileges of the Treasurer shall be as follows:

(a) He shall be in charge of the funds of the Society.

(b) At the Annual Business Meeting of the Society he shall present a statement to date of the funds of the Society.

(c) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society.

AUDITING COMMITTEE

(4) The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Treasurer at the meeting for which they were appointed.

(5) The National Research Council allows the Society three representatives on the Division of Biology and Agriculture. Of these three representatives, one shall be elected each year to serve three years. The method of election shall be the same as that used in the election of the officers of the Society.

MEETINGS

(6) It shall be the policy of the Society to hold meetings in both Eastern and Central-Western territory, and the distribution of the meetings between the two territories shall be determined in general on the basis of the representation of Eastern and Western members in the Society. See also 2-b.

PROGRAM RULES

(7) In matters relating to programs for annual meetings the following rules shall be observed:

(a) Papers shall be listed and presented according to subject matter in the following groups: 1. Comparative Anatomy; 2. Embryology; 3. Cytology; 4. Genetics; 5. Comparative and General Physiology; 6. Ecology, and 7. Miscellaneous, or other groups at the discretion of the Secretary.

(b) Whenever conditions require it the Executive Committee shall schedule two or more groups for the same hour and rearrange the program to bring together papers on subjects of more general interest for meetings of the whole Society. The Committee, however, is instructed to avoid conflicts as much as possible.

(c) Papers shall be listed in their respective groups in the order received. Each active member is entitled to fifteen minutes of the time of the Society at the annual meeting which he may use to present one or more papers personally, or he may use any or all of this time in introducing papers of non-members on the program. The Society recognizes, however, the right of the Genetics Section to make their own program rules in conjunction with the Genetics Section of the Botanical Society of America.

(d) All papers not read when called for as listed shall be placed at the end of the group list, and, if not read when called for the second time, they shall be read by title only.

(e) The titles of "introduced" papers shall be listed in the groups after the titles of papers to be read by members. Such papers shall be read by title only in case the entire program cannot be completed during four regular sessions for reading papers.

(f) Fifteen minutes shall be the maximum time allowed for the presentation of a paper.

(g) Abstracts of papers for publication in the proceedings of the Society must be handed to the Secretary or his representative before final adjournment of the annual meeting.

ASSOCIATE MEMBERS

(8) Associate members may become members of sections of the Society and are entitled to the journal privileges of members.

HISTORICAL REVIEW

A review of the historical antecedents of the present American Society of Zoölogists will be found in *The Anatomical Record* for January, 1917. The list of officers and meeting places of the present Society found in the same place is brought up to date and reprinted here.

LIST OF FORMER OFFICERS

American Morphological Society

President	*Vice-President*	*Secretary-Treasurer*
1890—E. B. Wilson	.....	J. P. McMurrich
1891—C. O. Whitman	E. L. Mark	J. P. McMurrich
1892—C. O. Whitman	H. F. Osborn	J. P. McMurrich
1893—C. O. Whitman	E. B. Wilson	J. P. McMurrich
1894—C. O. Whitman	W. B. Scott	G. H. Parker
1895—E. B. Wilson	W. B. Scott	G. H. Parker
1896—E. L. Mark	H. F. Osborn	G. H. Parker
1897—C. S. Minot	S. I. Smith	G. H. Parker
1898—H. F. Osborn	T. H. Morgan	G. H. Parker
1899—E. G. Conklin	W. M. Wheeler	Bashford Dean
1900—T. H. Morgan	H. C. Bumpus	J. S. Kingsley
1901—J. S. Kingsley	E. A. Andrews	T. H. Montgomery
1902—H. C. Bumpus	G. H. Parker	M. M. Metcalf

Additional Members of the Executive Committee

1891—E. B. Wilson	1897—J. S. Kingsley
H. F. Osborn	Bashford Dean
1892—E. L. Mark	1898—C. B. Davenport
T. H. Morgan	F. R. Lillie
1893—T. H. Morgan	1899—J. P. McMurrich
C. B. Davenport	G. H. Parker
1894—E. A. Andrews	1900—F. R. Lillie
F. H. Herrick	Jacob Reighard
1895—T. H. Morgan	1901—C. F. W. McClure
S. Watase	C. W. Hargitt
1896—E. G. Conklin	1902—H. S. Jennings
William Patten	R. G. Harrison

American Society of Zoölogists

EASTERN BRANCH	*President*	CENTRAL BRANCH
G. H. Parker	1903	Jacob Reighard
E. A. Andrews	1904	C. H. Eigenmann
W. E. Castle	1905	F. R. Lillie
W. E. Castle	1906	C. C. Nutting
C. B. Davenport	1907	S. A. Forbes
W. M. Wheeler	1908	E. A. Birge
H. S. Jennings	1909	E. A. Birge
T. H. Montgomery	1910	C. E. McClung
H. V. Wilson	1911	George Lefevre
A. G. Mayer	1912	H. B. Ward
Raymond Pearl	1913	H. B. Ward

LIST OF FORMER OFFICERS

EASTERN BRANCH	*Vice-President*	CENTRAL BRANCH
Jacob Reighard	1903	H. F. Nachtrieb
W. E. Castle	1904	S. J. Holmes
William Patten	1905	William A. Locy
William Patten	1906	George Lefevre
F. H. Herrick	1907	H. B. Ward
H. S. Jennings	1908	M. F. Guyer
H. V. Wilson	1909	M. F. Guyer
H. H. Wilder	1910	H. F. Nachtrieb
H. E. Crampton	1911	R. H. Walcott
G. A. Drew	1912	C. M. Child
Alex. Petrunkevitch	1913	C. M. Child
	Secretary-Treasurer	
G. A. Drew	1903	Frank Smith
G. A. Drew	1904	F. R. Lillie
H. S. Pratt	1905	C. E. McClung
H. S. Pratt	1906	T. G. Lee
C. J. Herrick	1907	T. G. Lee
L. L. Woodruff	1908	T. G. Lee
L. L. Woodruff	1909	Charles Zeleny
H. W. Rand	1910	H. V. Neal
Raymond Pearl	1911	H. V. Neal
J. H. Gerould	1912	W. C. Curtis
Caswell Grave	1913	W. C. Curtis

Executive Committeemen

F. R. Lillie	George Lefevre
T. H. Montgomery	T. G. Lee
H. C. Bumpus	Herbert Osborn
H. S. Jennings	C. H. Eigenmann
E. A. Andrews	J. G. Needham
W. R. Coe	S. J. Holmes
G. A. Drew	W. A. Locy
M. M. Metcalf	C. M. Child
D. H. Tennent	R. H. Walcott
R. G. Harrison	W. C. Curtis
H. E. Jordan	Oscar Riddle
C. E. McClung	H. B. Ward
	Chauncey Juday
	H. W. Norris
	C. E. McClung
	H. F. Nachtrieb

AMERICAN SOCIETY OF ZOÖLOGISTS (AMALGAMATED)

	President	*Vice-President*	*Executive Committeemen*
1914.	C. E. McClung	M. F. Guyer	H. E. Jordan—1 year H. F. Nachtrieb—2 years H. V. Wilson—3 years George Lefevre—4 years A. F. Shull—5 years
1915.	W. A. Locy	W. E. Ritter	D. H. Tennent
1916.	D. H. Tennent	Charles Zeleny	R. P. Bigelow—5 years L. J. Cole—4 years
1917.	M. M. Metcalf	Charles Zeleny	H. V. Wilson
1918.	George Lefevre	L. L. Woodruff	M. M. Metcalf
1919.	C. M. Child	H. H. Wilder	George Lefevre
1920.	Gilman A. Drew	Caswell Grave	C. M. Child
1921.	Charles A. Kofoid	Aaron L. Treadwell	G. A. Drew
1922.	H. H. Wilder	Bennet M. Allen	C. A. Kofoid
1923.	M. F. Guyer	Robert A. Budington	H. H. Wilder
1924.	R. G. Harrison	R. K. Nabours	M. F. Guyer
1925.	C. R. Stockard	C. R. Moore	R. G. Harrison
1926.	S. O. Mast	W. C. Allee	C. R. Stockard
1927.	S. J. Holmes	Robert Chambers, Jr.	S. O. Mast
1928.	Caswell Grave	M. H. Jacobs	
1929.	C. B. Davenport	Fernandus Payne	

Secretary-Treasurer

1914–1918. Caswell Grave

1918–1921. W. C. Allee

Secretary	*Treasurer*
1921–1924. W. C. Allee	1922. D. H. Tennent
1925–1928. D. E. Minnich	1923–1924. R. H. Bowen
1928–1929. L. B. Arey	1925–1930. L. B. Arey
1929–1930. D. E. Minnich	

Representative in the Division of Biology and Agriculture, National Research Council

M. F. Guyer 1919–1921	G. H. Parker 1919–1922	F. R. Lillie 1919–1923
William Patten 1921–1924	H. S. Jennings 1922–1925	E. G. Conklin 1923–1926
J. T. Patterson 1924–1927	L. L. Woodruff 1925–1928	

Associate Editors of the Journal of Morphology

1921.	Gary N. Calkins	J. S. Kingsley	William Patten
1921–1922.	E. G. Conklin	M. F. Guyer	W. M. Wheeler
1921–1923.	C. A. Kofoid	F. R. Lillie	J. T. Patterson
1922–1924.	G. A. Drew	H. V. Neal	L. L. Woodruff
1923–1925.	Caswell Grave	D. H. Tennent	H. V. Wilson
1924–1926.	Helen Dean King	S. O. Mast	A. A. Schaeffer
1925–1927.	R. W. Hegner	Fernandus Payne	H. B. Ward
1926–1928.	L. J. Cole	J. H. Gerould	M. H. Jacobs
1927–1929.	W. C. Curtis	J. Percy Moore	A. F. Shull

LIST OF PLACES OF MEETING

American Morphological Society

1890—Boston
1891—Philadelphia
1892—Princeton
1893—New Haven
1894—Baltimore
1895—Philadelphia
1896—Boston
1897—Ithaca
1898—New York
1899—New Haven
1900—Baltimore
1901—Chicago
1902—Washington

Central Naturalists

1899—Chicago
1900—Chicago

Society of American Zoölogists

1901—Chicago
1902—Washington

American Society of Zoölogists

EASTERN BRANCH

1903—Philadelphia
1904—Philadelphia
1906—New York
1907—New Haven
1909—Boston
1910—Ithaca

JOINT MEETINGS

1905—Ann Arbor
1908—Baltimore
1911—Princeton
1912—Cleveland
1913—Philadelphia

CENTRAL BRANCH

1903—St. Louis
1905—(Mch.) Chicago
1907—(Mch.) Madison
1907—Chicago
1910—(Apr.) Iowa City
1910—Minneapolis
1912—(Apr.) Urbana

MEETING PLACES

1914—Philadelphia
1915—Columbus
1916—New York
1917—Minneapolis
1918—Baltimore
1919—St. Louis
1920—Chicago
1921—Toronto
1922—Boston
1923—Cincinnati
1924—Washington
1925—New Haven
1926—Philadelphia
1927—Nashville
1928—New York
1929—Des Moines

AMERICAN SOCIETY OF ZOÖLOGISTS

Officers for 1930

President.....H. V. Neal
Vice-President.....E. E. Just
Secretary.....D. E. Minnich
Treasurer.....L. B. Arey

Executive Committee

C. R. Stockard.....1930
S. O. Mast.....1931
S. J. Holmes.....1932
Caswell Grave.....1933

Officers of Genetics Section

Chairman.....L. J. Cole
Secretary-Treasurer.....P. W. Whiting
Society Representative.....L. J. Stadler
Representative on Editorial Board of "Journal of Heredity".....M. Demerec
Representative on "Biological Abstracts".....F. B. Hanson

Representative of the Society in the Division of Biology and Agriculture of the National Research Council

H. S. Jennings.....1932
L. C. Dunn, Alternate.....1932

Representative of the Society on the Type Culture Committee of the National Research Council

C. A. Kofoid.....1932

Representatives of the Society on the Council of The American Association for the Advancement of Science

P. W. Whiting H. H. Newman

Representatives of the Society on the Council of the Union of American Biological Societies

Ross G. Harrison Frank R. Lillie

Representatives of the Society on the Advisory Editorial Board of "Biological Abstracts"

H. S. Pratt, G. K. Noble.....*Taxonomy*
D. H. Tennent.....*General Embryology*
M. H. Jacobs.....*General Physiology*

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NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

DEVELOPMENTAL ANATOMY, A Textbook and Laboratory Manual of Embryology, by Leslie Brainerd Arey. Second edition. 1930. Size, $6\frac{1}{2} \times 10$ inches. Pages ix + 563. 532 illustrations, many of which are in color. Index. Cloth. Price, $6.50. W. B. Saunders Company, Philadelphia.

Since the book bears the same title and follows the general plan as a previous book by the same author, it is called 'Second Edition,' whereas it is practically a new work, each chapter having been replanned and rewritten. All fundamental advances in the science of embryology have been incorporated and the entire field revaluated. A feature particularly to be commended is the full discussion of teratology and teratogenesis, since these subjects are found nowhere else in the medical course. Besides the chapter on teratology, which includes both normal and abnormal twinning, the various chapters describe and picture specific anomalies. A chart or reference table shows at a glance the size and the comparative development of the body form, features, and systems of the fetus at ages ranging from three weeks to full term. The three parts of the book are General Development, Organogenesis, and a Laboratory Manual of Embryology. Each chapter is followed by a list of references cited.

THE LIFE OF INLAND WATERS, by James C. Needham and J. T. Lloyd. 1930. Size, 6×9 inches. 438 illustrations. Index. Bibliography. Cloth. Price, $3.50. Charles C. Thomas, Springfield and Baltimore.

An interesting and non-technical story of the plant and animal inhabitants of our fresh-water streams and lakes, emphasizing the ecological side of limnology while touching briefly on economic, aesthetic, educational, sanitary, social, and civic interests. In a small book on so large a subject the discussion is necessarily limited, but it gives a general view of the field and serves as an introduction and a stimulus to further research in specialized branches. The bibliography lists books for reference and for continuing the study of hydrobiology in any of its branches. The chapter headings are: Introduction, The Nature of Aquatic Environment, Types of Aquatic Environment, Aquatic Organisms, Adjustment to Conditions of Aquatic Life, Aquatic Societies, Inland Water Culture.

AN INTRODUCTION TO HUMAN EXPERIMENTAL PHYSIOLOGY, by F. W. Lamb. Foreword by A. V. Hill. 1930. Size, $5\frac{1}{2} \times 8\frac{3}{4}$ inches. Pages xii + 335. 59 illustrations. Bibliographies. Index. Fabrikoid. Price, $4.00. Longmans, Green & Co., New York.

A course of experiments on blood, respiration, and circulation which may be carried out by the student or demonstrated to him. It aims to give him an insight into the objects and purposes of each experiment and a grasp of physiological problems rather than to instruct him in laboratory technique. The first section of the course is devoted to the study of the physicochemical and biological properties of the student's own blood. It teaches how to draw out blood from the veins and how to test it for specific gravity, viscosity, coagulation, blood grouping, etc. The second section treats of respiration, and the third, of circulation. The use of the student as his own subject for experimentation impresses vividly the clinical value of laboratory work.

TEXTBOOK OF HUMAN HISTOLOGY AND MICROSCOPIC ANATOMY, by Johannes Sobotta. Translated from the fourth German edition by William Hunter Piersol. 1930. Volume I, Text-book. Size, $8 + 9\frac{3}{4}$ inches. Pages xiii + 345. 42 illustrations. Index. Buckram.

ATLAS OF HUMAN HISTOLOGY AND MICROSCOPIC ANATOMY, by Johannes Sobotta. Edited from the fourth German edition by William Hunter Piersol. Volume II, Atlas. 1930. Size, $8 \times 9\frac{3}{4}$ inches. 92 pages, consisting of explanations of the 535 illustrations on 68 colored and 24 uncolored plates from original drawings by W. Freytag. Buckram. Price for the set of two volumes, $14.00. G. E. Stechert & Co., New York.

The English translation follows exactly the lines of the latest German edition, both as to contents and manner of presentation. It is an exposition of the recognized facts of histology, with brief mention of the more important unsolved problems. A number of schematic drawings are found in the text-book, but for the representation of the actual appearance of the structures described one must consult the plates of the atlas. Marginal notes in the text-book refer to the plate numbers of the atlas. Section A of the text-book is concerned with the cell and its elements, the blood, and the various tissues—epithelial, connective, endothelial. Section B describes the microscopic anatomy of the organs grouped together in systems. There is also a chapter explaining the use and purpose of the microscope. The atlas and the text-book are one book in two volumes. They are not sold separately.

A MANUAL OF EXTERNAL PARASITES, by Henry Ellsworth Ewing. 1929. Size, $6 \times 8\frac{3}{4}$ inches. Pages xvi + 226. 96 illustrations. Index. Fabrikoid. Price, $4.50. Charles C. Thomas, Springfield and Baltimore.

Five major groups of parasites, the mites, the ticks, the biting lice, the sucking lice, and the fleas, are examined as to their classification, anatomy, habits, life history, and the methods by which they may be controlled. Each chapter has its list of references and descriptive keys for identification of the genera. As a text-book for biological students it is excellent and as a reference book for general practitioners, dermatologists, veterinarians, and growers of stock and poultry it is invaluable. The clear and concise descriptions and the numerous illustrations make possible the recognition of specific pests, and the methods of control as outlined furnish a means of combating them.

ANIMAL LIFE OF YELLOWSTONE NATIONAL PARK, by Vernon Bailey. 1930. Size, $5\frac{1}{2} \times 8\frac{1}{2}$ inches. 70 illustrations (four in color). Pages xii + 232. Cloth. Price, $4.00. Charles C. Thomas, Springfield and Baltimore.

A wide variety of animal life characteristic of four life zones flourishes in the park protected from hunters and, when necessary and possible, from starvation. The historic American bison, threatened with extinction in 1901 when only twenty-five specimens could be found, has multiplied until the combined herds of the park number more than one thousand. The bears, which roam at large, attract much attention from tourists. The author of this book says bears may be observed and photographed safely at close range if they are treated with respect and shown no

undue familiarity. Besides the large and spectacular animals, such as bears, buffaloes, moose, and elk, there are many other mammals, large and small, birds, fish, reptiles, amphibians, and invertebrates, some of them easily seen by visitors, others so shy that they are rarely seen at all. Naturalists, nature lovers, and students will find this book rich in details of animal habits and idiosyncrasies, and all visitors to the park, past, present, or prospective, should have a copy of it.

THE ANIMAL MIND, by C. Lloyd Morgan. 1930. Size, $5\frac{1}{2} \times 8\frac{3}{4}$ inches. Pages xii + 275. Index. Cloth. Price, $4.25. Longmans, Green & Co., New York.

A broad discussion of the animal mind in the general scheme of things; a leisurely piece of writing which avoids, on the one hand, mere anecdotal descriptions of surprising occurrences and, on the other hand, a too detailed array of observational data with the inferences drawn by the psychologists. It is addressed to the 'Gentle Reader' to whom the author assumes the years have brought the philosophic mind, and it gossips along the way about the universe and the queerness of human nature. Some of the biological, physiological, and psychological questions among the problems considered are levels of mentality, instinctive behavior, the dawn of intelligence, association, trial and error, memory, and the ignorance of animals. The final chapter is an inquiry into the question, Are Animals Automata?

BALANCE OF BIRTHS AND DEATHS, Volume I, Western and Northern Europe, by Robert R. Kuczynski. 140 pages. Size, $5 \times 7\frac{1}{2}$ inches. Index. Cloth. Price, $2.00. Published for The Brookings Institution by The Macmillan Company, New York.

The first volume of a series giving a comprehensive survey of the trend of human fertility and indicating the countries whose population is still amply reproducing itself and those whose population is ceasing to maintain itself. It analyzes statistics bearing on birth rates, fertility rates, net reproduction rates, present and future balance. The four appendices supply further data on births and birth rates, women of child-bearing age, age of mothers, life tables, and fertility tables.

A DICTIONARY OF GREEK AND LATIN COMBINING FORMS USED IN ZOÖLOGICAL NAMES, by Edmund C. Jaeger. 1930. Size, $4\frac{1}{4} \times 7\frac{1}{4}$ inches. 101 pages. Cloth. Price, $2.00. Charles C. Thomas, Springfield and Baltimore.

A practical book which makes intelligible the bewildering scientific names encountered in zoölogical study. The combining forms are given with their most commonly used endings, and each definition shows why the term is applicable to the animal, as, for instance, the Chiroptera, or hand-winged bats, are so called from the Greek *Chiro*, meaning hand. From the stem *phago*, meaning to eat, we get phagocyte, a white blood cell capable of devouring pathogenic cells or organisms. It is not exhaustive, but there are more than twelve hundred Greek and Latin terms commonly met with. The instructor will find it of help in his explanations, and the student can learn from it the fascination of the study of names.

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